Volume 15 MARCH-APRIL, 1950 Number 2

Food Research

Under the Editorial Guidance of

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Heureux déjà d' avoir pu servir mes concitoyens et l' humanite, je me repose sur la justice, la générosité et les lumières d' un gouvernement sage qui ne cesse d' encourager et de protéger toutes les découvertes utiles.

—NICOLAS APPERT.

Published Six Times a Year by THE GARRARD PRESS, Champaign, Illinois

Made in the United States of America

Food Research

FOOD RESEARCH is an independent journal founded for advancement and dissemination of scientific knowledge regarding the field of foods. It is devoted to publication of results of original investigations and occasional reviews, in the fields of food and beverage technology, chemistry, and microbiology. The services of all members of the Editorial Board are given with this desire and with no financial return.

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Manuscripts should be sent to the Editor-in-Chief, F. W. Tanner, 921 Lincoln Circle, Winter Park, Florida, or other members of the Board of Editors.

FOOD RESEARCH is issued bimonthly. Six issues constitute a volume.

Subscription price: Domestic, $5.50 per year; to foreign countries, $6.00 per year. Single copies of current number, one dollar; to England, 4s. All subscriptions should be sent to the publishers or their agent.

Claims for copies lost in the mails must be received within thirty days (ninety days, foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

New subscriptions and renewals are entered to begin with first issue of current volume.

All correspondence concerning advertising, reprints, orders for copies should be addressed to The Garrard Press, Champaign, Illinois.

European Agents
H. K. LEWIS & CO., LTD.
136 Gower Street
LONDON, W. C. 1, ENGLAND

THE GARRARD PRESS

Division of Twin City Printing Co.

Manufacturers and Publishers of Scientific Books and Periodicals

CHAMPAIGN, ILLINOIS, U. S. A.

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EDITORS

FRED W. TANNER
921 Lincoln Circle, Winter Park, Florida

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Cambridge, Massachusetts

L. B. JENSEN
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Chicago 9, Illinois

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CONTENTS

CAROLYN WRINKLE, H. H. WEISER, AND A. R. WINTER. Bacterial Flora of Frozen Egg Products 91

ARTHUR D. HOLMES. Darkness Prolongs Storage Life of Butternut Squashes 99

CONRADO F. ASENJO AND CARLOS G. MOSCOSO. Ascorbic Acid Content and Other Characteristics of the West Indian Cherry 103

D. I. MURDOCK. Inhibitory Action of Citric Acid on Tomato Juice Flat-Sour Organisms 107

EDWARD A. NEBESKY, WILLIAM B. ESSELEN, JR., A. M. KAPLAN, AND CARL R. FELLERS. Thermal Destruction and Stability of Peroxidase in Acid Foods 114

MARY CATHERINE WETHINGTON AND F. W. FABIAN. Viability of Food-Poisoning *Staphylococci* and *Salmonellae* in Salad Dressing and Mayonnaise 125

F. W. FABIAN AND MARY CATHERINE WETHINGTON. Spoilage in Salad and French Dressing Due to Yeasts 135

F. W. FABIAN AND MARY CATHERINE WETHINGTON. Bacterial and Chemical Analyses of Mayonnaise, Salad Dressing, and Related Products 138

T. L. WARD, W. S. SINGLETON, AND A. F. FREEMAN. Heat Capacity of Stabilized Peanut Butter 146

A. E. STEVENSON. Rutin Content of Asparagus 150

HELEN CHARLEY. Effect of Baking Pan Material on Heat Penetration During Baking and on Quality of Cakes Made With Fat 155

ROYENE F. OWEN AND FRANCES O. VAN DUYNE. Comparison of the Quality of Freshly Baked Cakes, Thawed Frozen Baked Cakes, and Cakes Prepared From Batters Which Had Been Frozen 169

Published bimonthly by The Garrard Press, division of Twin City Printing Co., Champaign, Illinois. Domestic subscription, $5.00 per year; to foreign countries $6.00. Entered as second class matter at the Post Office at Champaign, Illinois, under the act of March 3, 1879.

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BACTERIAL FLORA OF FROZEN EGG PRODUCTS[1]

CAROLYN WRINKLE[2] AND H. H. WEISER
Department of Bacteriology
AND
A. R. WINTER
Department of Poultry Husbandry, Ohio State University, Columbus, Ohio

[Received for publication, October 16, 1949]

Marketing of eggs in the form of frozen egg products is an important and growing business. According to estimates of the United States Department of Agriculture (1949), more than 300 million pounds of frozen egg products are produced annually, with a value of more than 80 million dollars.

The bacterial flora of frozen egg products is important, because it influences the wholesomeness of the products, their keeping quality, and their functional properties in the preparation of food. Haines (1939), Gibbons, Moore, and Fulton (1944), Winter, Stewart, McFarlane, and Solowey (1946), and others have presented data which indicate that more than 80 per cent of freshly laid eggs are bacteriologically sterile. However, nearly all commercial liquid, frozen, and dried egg products contain several hundred to several million bacteria per gm., according to reports by Redfield (1920), Johns (1944), McFarlane, Watson, Sutton, and Kurman (1947), Winter and Wrinkle (1949), and others.

Dirt on egg shells and practices used in egg-breaking plants account for most of the bacteria found in liquid, frozen, and dried egg products, according to Haines (1939), Rosser (1942), Stuart and McNally (1943), Zagaevsky and Lutikova (1944), McFarlane, Watson, and Goresline (1945), Johns and Berard (1945), Gunderson and Gunderson (1945), Solowey, Spaulding, and Goresline (1946), Penniston and Hedrick (1947), and Winter and Wrinkle (1949). A very heterogeneous and variable flora may be expected in frozen egg products, since dirt on the shell consists of fecal material, soil, nest and feather dust, and microorganisms in the air, on equipment and on workers in the breaking plants.

Haines (1939) has reviewed the early literature dealing with microflora on and in shell eggs. His own investigations showed the percentage distribution of microflora in washings from shell eggs to be non-spore-forming rods 38, sporing rods 20, *cocci* 25, yeasts 4, and molds 3. Since then Zagaevsky and Lutikova (1944) and Gunderson and Gunderson (1945) have reported somewhat similar flora on egg shells. Hartsell (1944), The Medical Research Council of Great Britain (1947), and McFarlane and Calesnich (1948) have reported *enterococci* and aerobic spore-forming *bacilli* as the bacteria found most frequently and in greatest numbers in dried egg. *Escherichia coli*, *Salmonella*, and molds were also frequently found in dried eggs.

[1] This study was made possible by a grant from the Refrigeration Research Foundation to the Ohio State University Research Foundation.

[2] Now with Tennessee Valley Authority, Knoxville, Tennessee.

Swenson and James (1935), Nielsen and Garnatz (1940), Schneiter, Bartram, and Lepper (1943), and Winter and Wrinkle (1949) have shown that freezing and storage reduce the viable bacteria counts in egg products. McFarlane *et al.* (1945), Winter, Creco, and Stewart (1946), Gibbons, Fulton, and Reid (1946), and the Egg Producers' Council of Australia (1948) have also shown that pasteurization of egg magma greatly reduces the viable bacteria counts. However, very few data are available on the composition of bacterial flora in egg magma, and practically none on qualitative and quantitative changes resulting from pasteurization and/or freezing and storage.

Objectives of this investigation have been: (1) To classify the viable bacteria found in egg magma into groups based on (differential) culture media. (2) To determine the effect of pasteurization and freezing on changes in the species and percentage distribution present. (3) To determine the effects of species of bacteria, isolated from egg, when inoculated into bacteriologically sterile liquid whole egg and incubated at room temperature.

EXPERIMENTAL PROCEDURE

Egg Samples: Large samples of liquid whole egg, white, and yolk were obtained from a local egg-breaking plant. Some samples were also obtained by breaking out dirty and cracked eggs obtained from the university flock. Freshly prepared egg samples of high bacterial count were desired, in order to cover the general range of flora and to be able to show effects of pasteurization and freezing on changes in flora.

Bacteriologically sterile samples of eggs were obtained by soaking fresh, clean eggs in five per cent sodium hydroxide; draining; dipping in 95 per cent alcohol; flaming; breaking into a sterile hand-homogenizer under a hood provided with germicidal lamps; and homogenizing into a sterile jar. Samples were transferred aseptically to culture tubes for inoculation.

Bacterial Analysis: Liquid, frozen, and defrosted egg products were sampled for analysis according to the method of the Association of Official Agricultural Chemists (1945). Standard dilution and plating techniques were used. All samples were plated in triplicate at each dilution used.

Standard plate counts were made on tryptone glucose extract agar (Difco). The plates were incubated at 32°C.(90°F.) for 72 hours. The percentage distribution of different types of surface colonies was determined. Organisms from each type of colonies were isolated and identified according to Bergey's Manual (1948). Typical cultures were preserved on stock culture media for inoculation into bacteriologically sterile egg.

Similar groups of tryptone glucose extract agar plates were incubated at 55°C.(131°F.) for 48 hours, to determine the thermoduric and thermophilic nature of the flora.

Gram-positive *cocci* were counted on crystal violet agar, containing a one to 5,000 concentration of sodium azide, after 48 hours' incubation at 37°C.(99°F.). The media permits the growth of gram-positive *cocci*, especially *Streptococcus faecalis*, while inhibiting the growth of gram-negative bacteria according to Packer (1943) and Chapman (1944).

Gram-negative coliforms (lactose fermenters) were counted on desoxycholate (RBL) agar after 18 to 24 hours' incubation at 37°C., according to the method of Leifson (1935).

The gram-negative, non-lactose fermenters which comprised the enteric, possibly pathogenic, bacteria were incubated on bismuth sulfite agar (Difco) for 48 hours at 37°C.

Pasteurization: Liquid egg samples were pasteurized in an experimental laboratory pasteurizer according to the method of Winter *et al.* (1946). Each sample was thoroughly mixed and divided into two parts. One part was used as the unpasteurized control, while the other was pasteurized at a definite temperature and for a given period of time.

Freezing and Storage: Samples were packaged in sterile pint freezer jars, frozen at temperatures ranging from −17 to −23°C.(2 to −10°F.) and stored at the same temperatures for periods ranging from one to 21 days.

Defrosting and Spoilage: After the frozen samples were sampled for analysis, the containers were held at room temperature and observed morning and night until spoilage occurred. This usually took place within 24 hours after the samples were completely defrosted. Spoilage was judged mainly by the development of an abnormal odor. Consistency and color changes were also considered in judging spoilage.

RESULTS

Distribution of bacterial flora was determined before and after pasteurization; before and after freezing; and after defrosting and spoilage in 27 samples of liquid egg products. Distribution of flora in eight typical samples is shown (Table 1). In high count samples, coliforms and gram-positive *cocci* were the principal organisms found. They probably came from dirt on the shell, chiefly of fecal origin.

Pasteurization of whole eggs at 61 to 62°C.(143 to 144°F.) for 3.7 to four minutes killed more than 99 per cent of the standard plate count bacteria, nearly all of the coliforms and gram-positive *cocci*, and all of the possibly pathogenic enteric bacteria in the three samples of the whole egg. Egg white cannot be pasteurized above 56 to 57°C.(132 to 134°F.) without coagulation difficulties. Pasteurization of the sample of white at 56°C.(132°F.) for four minutes resulted in the destruction of 92 per cent of the standard plate count bacteria.

Freezing and storage of both unpasteurized and pasteurized samples resulted in destruction of bacteria. However, the destructive effect of freezing at −22 to −23°C.(−7 to −10°F.) and storage at the same temperature for from two to three weeks was not nearly as great as pasteurization at 61 to 62°C.(143 to 144°F.) for 3.7 to four minutes. This was true for the differential media counts of bacteria, as well as for the standard plate counts. Exceptions (observed Table 1) may be due to errors in sampling. These become more marked in the case of high count egg magma, possibly because of small particles of dirt scattered unevenly throughout the egg cans and jars from which samples were taken. Shaking of the samples in the presence of glass beads would disintegrate the small particles of dirt, usually fecal material, and thus liberate many bacteria.

TABLE 1

Influence of Pasteurization, Freezing and Storage, Defrosting, and Holding Liquid Egg Products on Changes in Their Bacterial Flora[1]

Sample and treatment	Standard plate count	Thermodurics and thermophiles	Coliforms gram—lactose+	Enterics gram—lactose—	Gram+ cocci (*Strep. faecalis*)
1. Whole egg					
Unpasteurized					
Before freezing	3,890	0.423	108.7	0.54	2.57
After freezing —10°F., 13 days	3,300	27	89.67	0.49	0.13
Change (pct.)	—15	6,283	—18	—9	—95
Defrosted and spoiled	398,000	0.647	5,400	0.04	29.3
Pasteurized 144°F., 3.7 min.					
Before freezing	0.27	0.023	0.007	0.0	0.189
After freezing	0.077	0.006	0.0	0.0	0.02
Change (pct.)	—71	—74	—100	0.0	—89
Defrosted and spoiled	160,000	2.7	0.191	0.04	0.171
2. Whole egg					
Unpasteurized					
Before freezing	1,730	0.017	0.01	0.0	591
After freezing —7°F.,14 days	99	0.0	0.006	0.0	78
Change (pct.)	—94	—100	—40	0.0	—87
Defrosted and spoiled	518,000	0.04	7	0.0	465,000
Pasteurized 144°F., 4 min.					
Before freezing	1.3	0.19	0.0	0.0	0.0
After freezing	0.18	0.0	0.0	0.0	0.0
Change (pct.)	—86	—100	0.0	0.0	0.0
Defrosted and spoiled	241,000	0.0	0.0	0.0	32.76
3. Whole egg					
Unpasteurized					
Before freezing	7,470	0.39	73.8	0.008	2,610
After freezing 2°F.,21 days	10,700	0.07	45	0.006	1,180
Change (pct.)	43	—82	—37	—25	—55
Defrosted and spoiled	1,630,000	0.005	360,000	0.0	1,900,000
Pasteurized 143°F., 4 min.					
Before freezing	1.7	0.046	0.033	0.0	0.65
After freezing	1.56	0.0	0.003	0.0	0.80
Change (pct.)	+33	—100	—91	0.0	39
Defrosted and spoiled	35,000	0.5	0.0	0.0	28,000
4. White					
Unpasteurized					
Before freezing	27.43	0.025	0.053	0.015	0.09
After freezing —7°F., 7 days	4.6	0.017	0.05	0.0	0.023
Change (pct.)	—83	—32	—6	—100	—75
Defrosted 24 hr.	834	0.0	0.493	0.03	0.0
Defrosted 96 hr.	138,000	17.0	6,570	0.5	0.0
Pasteurized 132°F., 4 min.					
Before freezing	2.05	0.025	0.01	0.0	0.05
After freezing	13	0.017	0.0	0.0	0.0
Change (pct.)	534	—32	—100	0.0	—100
Defrosted 24 hr.	356	0.037	0.0	0.0	0.0
Defrosted 96 hr.	97,000	100	0.7	0.618	0.0
5. White					
Before freezing	11.97	0.103	4.88	0.013	0.007
After defrosting	46	0.0	0.33	0.023	0.02
Change (pct.)	284	—100	—93	77	185
When spoiled	12,170	0.58	930	0.96	5.43
6. Yolk—plain					
Before freezing	165	0.215	0.05	2	0.3
After freezing 2°F., 24 hr.	12	0.020	0.04	3.5	0.05
Change (pct.)	—93	—91	—20	75	—83
7. Yolk with 10% salt					
Before freezing	13.07	0.013	2.61	0.077	0.05
After freezing —5°F., 18 days	3.73	0.027	0.12	0.07	0.047
Change (pct.)	—72	108	—95	—9	—6
Defrosted and spoiled	27	0.320	0.0	0.64	10
8. Yolk with 10% sugar					
Before freezing	16	0.09	0.05	1.7	0.55
After freezing 2°F., 24 hr.	180	0.015	0.085	6.0	0.1
Change (pct.)	1,025	—83	70	253	—82

[1] Thousands of bacteria per gram.

Distribution of genera of bacteria in seven samples of unpasteurized, and four pasteurized, samples of whole egg before and after freezing is shown (Table 2). *Alcaligenes, Flavobacterium, Proteus,* and *Pseudomonas* bacteria predominated among the 13 genera isolated most frequently in unpasteurized egg. Freezing reduced the number of bacteria but had little effect on number of genera present. Pasteurization reduced the number of genera present. The same genera of bacteria that predominated in unpasteurized egg also predominated in pasteurized egg.

TABLE 2

Distribution of Principal Genera of Bacteria in Whole Egg Samples

Genera	Unpasteurized		Pasteurized	
	Before freezing	After freezing	Before freezing	After freezing
	pct.	*pct.*	*pct.*	*pct.*
Achromobacter	0.0	1.5	.....	.....
Aerobacter	3.0	0.0	.....	.....
Alcaligenes	25.1	20.0	4.0	8.3
Bacillus	7.4	2.0	8.3	83.5
Chromobacter	1.6	1.6	.....	.....
Eberthella	1.0	0.0	.....	.....
Escherichia	5.7	6.8	2.5	0.0
Flavobacterium	29.0	26.9	4.0	0.0
Gram + *cocci*	5.3	3.5	2.5	0.0
Proteus	15.6	18.1	4.0	8.3
Pseudomonas	7.4	16.0	.....	.....
Salmonella	1.0	0.0	.....	.....
Streptothrex	0.0	2.3	.....	.....

When egg samples were defrosted and allowed to spoil, there was usually a great increase in number of coliforms or gram-positive *cocci*, but seldom a big increase in both groups in the same sample (see Table 1). An examination of the surface colonies on the tryptone glucose extract agar plates from spoiled samples showed that *Proteus, Alcaligenes, Escherichia,* and *Flavobacterium* were the predominating genera.

Cultures of several strains of the most common genera of bacteria found in liquid egg were inoculated into tubes of sterile egg. Observations were made on the changes produced; the data are summarized (Table 3). Most of the species or strains of *Alcaligenes* and *Escherichia*, the two most common bacteria found in liquid egg, produced a sour odor in egg within 60 hours after inoculation into bacteriologically sterile egg and incubation at room temperature. *Flavobacteria* produced slow changes in egg in most instances, which ranged from a slight odor to coagulation. *Proteus* bacteria produced souring and sometimes coagulation. *Pseudomonas* produced souring.

The fact that all inoculations of a given genera did not always produce the same reaction may have been due to the use of different species. Time did not permit a study of the microflora beyond groupings and genera identification.

Levine and Anderson (1932) and others have shown that *Achromobacter* and *Pseudomonas* may produce mustiness in eggs. Haines (1938) has shown that certain atypical strains of *E. coli* produce a turbidity and

TABLE 3

Changes Produced by Different Genera of Bacteria Isolated From Liquid Egg, When Inoculated Into Sterile Egg

Genus	No. different strains inoculated	Changes produced
Achromobacter	1	No change in 72 hr.
Aerobacter	4	Slight acid odor after 72 hr.
Alcaligenes	17	12 Very sour odor in 60 hr. 1 Musty odor at 48 hr. 4 No detectable change
Bacillus	8	6 Coagulation within 18 hr. 1 Very sour odor after 24 hr. 1 No change
Chromobacter	2	No change in 72 hr.
Escherichia	15	4 Slight acid odor in 60 hr. 8 Sour odor after 60 hr. 3 No detectable change
Flavobacterium	11	2 Coagulation in 120 hr. 1 Fecal odor at 72 hr. 4 Slight odor at 120 hr. 4 No detectable change at 72 hr.
Gram + *cocci*	3	2 Produced much gas in 18 hr. 1 No change
Proteus	10	3 Very flat sour odor in 60 hr. 2 Coagulation in 18 hr. 4 Very sour odor in 60 hr. 1 No change
Pseudomonas	7	3 Very sour odor in 60 hr. 2 Gas within 60 hr. 1 Sour odor in 60 hr. 1 No change

fishy flavor in egg white. He found that some species of *Pseudomonas* produced a "cabbage water" flavor in white. Winter (1942) and Funk (1943) have produced spoilage in shell eggs by inoculation with *Alcaligenes, Escherichia, Proteus,* and some other genera. Lorenz and Starr (1949) have shown that the souring of washed cold storage eggs is frequently due to *Pseudomonas.* More work should be done on the influence of bacteria on the keeping quality of eggs.

SUMMARY

Coliforms and gram-positive *cocci* were the principal bacteria found in high standard plate count samples of liquid egg.

Freezing at −17 to −23°C.(2 to −10°F.) and storage at the same temperatures for from two to three weeks reduced the numbers of bacteria but did not eliminate any of the groups of bacteria studied.

Pasteurization of liquid whole egg at 61 to 62°C.(143 to 144°F.) for 3.7 to four minutes reduced the standard plate count bacteria more than

99 per cent; nearly all the coliforms and gram-positive *cocci*; and all the possibly pathogenic gram-negative enteric bacteria.

Alcaligenes, Flavobacterium, Proteus, and *Pseudomonas* were the genera which comprised most of the bacteria found in liquid and frozen egg products. All of these organisms produced spoilage when inoculated into sterile liquid whole egg.

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DARKNESS PROLONGS STORAGE LIFE OF BUTTERNUT SQUASHES[1]

ARTHUR D. HOLMES
Massachusetts Agricultural Experiment Station, Amherst, Massachusetts

[Received for publication, November 2, 1949]

In many localities during the fall, a large tonnage of squashes is placed in storage for consumption in winter months. Ordinarily, the larger portion of the squashes is stored in darkness, in specially-constructed squash houses equipped with a heating system for use during periods of low atmospheric temperature. For a number of years a portion of the squash crop produced in Massachusetts has been stored in greenhouses which are provided with heat and are not used for growing plants during the early winter months. Obviously, squashes stored under such conditions are exposed to sunshine and skyshine, even though the winter storage period for squashes coincides with the season of minimum daylight. According to Stapleton (1948), the normal number of clear and fair days in this area during early winter furnishes 121 hours of bright sunshine in November, 128 in December, 137 in January, and 158 in February. In addition, skyshine contributes considerable light even during the winter months.

These observations naturally raised a question as to the effect of this amount of light upon the storage life of squashes. In a study of the influence of light during storage on the composition of Blue Hubbard squashes, Holmes, Spelman, Rogers, and Lachman (1948) found no consistent difference in the composition of Blue Hubbard squashes stored in the light and in the dark. Since the hard outer shell of mature Blue Hubbard squashes is rather impervious to the passage of light, a second test was made with Butternut squashes, which have a comparatively thin skin, and Holmes (1948) observed an 80 per cent loss through decay of squashes stored in the light, as compared with a 70 per cent loss of similar squashes stored in darkness. The present study was conducted to accumulate additional data regarding effect of light on storage life of Butternut squashes.

EXPERIMENTAL PROCEDURE

Squashes used in this study were produced under the carefully controlled conditions maintained in genetic studies designed to develop a commercially desirable strain of Butternut squashes. Each squash was carefully examined during selection of specimens for the storage study. All squashes were well-colored, fully matured, and free from any bruises or blemishes. In order to avoid any possible infection through a wound at the stem end, all squashes were cut from the vines and three to four inches of the vine remained on each squash throughout the experimental period (245 days). The squashes were transported from the field to the laboratory in lettuce boxes padded with newspapers. On arrival at the laboratory, 120 of the most perfect specimens were selected and divided

[1] Contribution No. 745, Massachusetts Agricultural Experiment Station.

into two comparable lots of 60 each. Both lots were stored in a dry, well-ventilated laboratory attic—one lot in darkness and the other in continuously controlled illumination. The two lots were about two feet apart and separated by a one-half-inch movable partition. Illumination was provided by four 20-inch daylight fluorescent electric bulbs, suspended beneath a 46- by 49-inch reflector 24 inches above the squashes, which were placed close together.

The experimental period was from September 23 to May 27, inclusive. Temperature of the storage space ranged from 13 to 22°C.(55 to 72°F.), but for the larger portion of the period was about 18°C.(64°F.). The maximum temperature occurred in the early and late portions of the experimental period, and the minimum in mid-winter. Relative humidity of the storage space varied from 36 to 72 per cent, as very temporary extremes. In general the relative humidity was approximately 50 per cent; in fact, frequently readings remained at 50 per cent for several days at a time. Holmes (1948) noted in a previous study that the storage life of Butternut squashes is relatively short as compared with that of the hard-shelled varieties, such as Blue Hubbard Squashes, so in this study the squashes were weighed and individually examined for evidences of spoilage at approximately two-week intervals. At these intervals all squashes that remained perfect in physical appearance were classified as "marketable"; those that showed any discoloration and/or any shriveling of the neck, but which were still firm and gave no detectable evidence of beginning spoilage, were classified as "edible."

RESULTS AND DISCUSSION

Detailed data regarding the storage life of Butternut squashes exposed to light and darkness during the winter months are included (Table 1). When placed in storage, the average weight of the 60 squashes kept in darkness was 999.1 gm., and the average weight of the 60 squashes exposed to continuous illumination was 997.5 gm. Loss in weight, five per cent, was largest during the first fortnight of storage, and was practically the same for both groups. In fact, at 14 of the 18 intervals at which the squashes were weighed individually, average weights for the two series were within one per cent of each other. Squashes stored in continuous illumination lost 38 per cent of their original weight during the 245-day storage period, as compared with a 36 per cent loss in weight of those stored in darkness. Thus, presence or absence of light seemed to have little or no effect upon rate or amount of loss in weight of Butternut squashes during winter storage. In the previous study of storage losses of Butternut squashes, Holmes (1948) reported a 15.8 per cent weight loss for squashes stored in continuous light for nine weeks, and 20.3 per cent loss for squashes stored in darkness; but in this study, losses were 17 and 15 per cent, respectively, for 70 days' storage. Phillips (1946) found a 25 per cent loss in weight during three months' storage, which was definitely larger than the 19 and 18 per cent that occurred in this study during 90 days' storage. In studies of Butternut squash storage losses Young (1947) noted a 29 per cent weight loss at the end of 14 weeks, which is materially larger than the 24

TABLE 1

Results of Butternut Squash Storage Studies

Length of storage	Storage in light				Storage in darkness			
	Quality		Storage losses		Quality		Storage losses	
	Marketable	Edible	Spoilage	Weight	Marketable	Edible	Spoilage	Weight
days	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
0	100	100	0	0	100	100	0	0
13	100	100	0	5	100	100	0	5
27	100	100	0	0	96	100	0	8
41	93	100	0	12	93	100	0	11
55	77	100	0	14	93	100	0	12
70	21	100	0	17	78	100	0	15
90	12	98	2	19	60	98	2	18
103	7	96	4	21	41	96	4	20
118	7	96	4	23	....	....	....	....
132	7	86	14	25	35	89	11	24
146	7	70	30	26	31	80	20	26
160	7	51	49	27	31	70	30	28
174	7	50	50	28	26	70	30	29
188	7	43	57	29	26	68	32	30
203	7	37	63	32	26	65	35	32
216	5	33	67	34	20	56	44	33
231	5	30	70	36	15	50	50	35
245	5	30	70	38	15	40	60	36

and 20 per cent weight loss noted here for squashes, stored under illumination and in darkness, respectively.

There were no losses from spoilage in either lot for 70 days, but for the remainder of the 245-day storage period, squashes were continually eliminated from each lot. Total loss from spoilage was 70 per cent for squashes stored in light and 60 per cent for squashes stored in darkness. However, black rot (*Mycosphaerella citrullina*) was much less prevalent, and the areas involved on individual squashes were quite small as compared with the conditions reported by Holmes (1948) in an earlier study. It has been suggested that, because of a drought at the period of the growing season when squashes are infected with black rot, squashes in the present study were not seriously infected.

As the storage period progressed, the number of squashes suitable for marketing decreased. At the end of six weeks' storage, seven per cent of the squashes in each group were considered as unsuited for marketing. From that time, the number of squashes that retained marketable quality decreased at a more rapid rate, particularly of the squashes stored under illumination. At the end of 90 days' storage, only 12 per cent of the squashes stored in the light and 60 per cent of the squashes stored in darkness were in marketable condition. Judged by these data, the commercial squash grower should not consider holding Butternut squashes in storage for three months before marketing them. However, these losses are much smaller than those reported by Holmes (1948) for the previous study, in which all the squashes had spoiled before the end of three months' storage. In this study, the number of marketable squashes at the conclusion of the 245-day experimental period was five per cent for the lot stored in

light and 15 per cent for those stored in darkness. Thirty per cent of the squashes stored in the light were edible at the termination of the study, and 40 per cent of those stored in darkness were still edible, although many of them showed some discoloration of the outer skin and/or some shriveling of the neck.

Results obtained under the conditions maintained in this study indicate that storing Butternut squashes in darkness is preferable to storing them in light. These results are in accord with those obtained in the previous study by Holmes (1948).

SUMMARY

Fully matured, well-colored, and carefully selected Butternut squashes grown under controlled conditions were divided into two comparable lots. One lot was stored for 245 days under continuous artificial illumination, and the second lot stored in darkness; both lots were exposed to the same temperature and other experimental conditions. Weight and spoilage losses were slightly larger for the squashes stored in the light than for those stored in darkness. At the end of the storage period, more of the squashes that had been stored in darkness than of those that had been stored in light were marketable.

ACKNOWLEDGMENT

The author is very grateful to Dr. O. H. Pearson, director of genetic investigations, Eastern States Farmers' Exchange, Field Station, Feeding Hills, Massachusetts, for supplying the squashes used in this study.

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ASCORBIC ACID CONTENT AND OTHER CHARACTERISTICS OF THE WEST INDIAN CHERRY

CONRADO F. ASENJO AND CARLOS G. MOSCOSO
Department of Chemistry and Nutrition, School of Tropical Medicine, and Department of Plant Breeding, Agricultural Experiment Station, University of Puerto Rico

[Received for publication, November 2, 1949]

The West Indian cherry (*Malpighia punicifolia* L.) is a small, bushy tree which grows to a height of from 10 to 15 feet. It is found in the tropical and semitropical regions of America.

The fruit of the tree is small, weighs approximately four gm., and varies from one-half inch to an inch in diameter. Its color ranges from a light-green, when the fruit is immature, to a yellowish-orange, or a deep, bright red, depending on the stage of maturity, variety of cherry, conditions of soil, and type of fertilizer used. The small seeds in each cherry fluctuate from five to six in number, depending on the variety of the fruit. Approximately 80 per cent of the weight of the fruit is edible.

Fortunately, this tree is extremely hardy; it adapts itself easily to poor soils, and can apparently resist most diseases caused by insect pests. It requires little attention other than at harvest time. In a mature tree, and depending on its variety, a harvest may be expected every 40 days to two months during the bearing season.

Varieties of the tree found so far have not yet been standardized, but experiments are now in progress which should permit the selection of outstanding varieties in the near future.

From observation of the trees in their wild state in various sections of Puerto Rico, it is apparent that the West Indian cherry thrives best on well-drained soils, or sandy loam with abundant rainfall. The size of the fruit and the quantity of the crop may be influenced by the amount of rain; the trees produce a larger and more palatable fruit during a rainy season.

Asenjo and Freire de Guzmán (1946) reported that the West Indian cherry contained from one to three gm. of ascorbic acid per 100 gm. of edible matter, and that the green fruit contained more of the acid than did the ripe. The writers obtained these values by using the 2,6-dichlorophenol indophenol titration, and definitely established the presence of this large amount of ascorbic acid by isolating the same in a crystalline form from the juice.

Later, Mustard (1946) confirmed the above findings when she analyzed several samples of West Indian cherry, grown in Florida, and reported values as high as 4,676 mg. of ascorbic acid per 100 gm. of edible matter of the unripe fruit. She also investigated the ascorbic acid content of several samples of jelly prepared from this cherry, always obtaining values above 450 mg. per 100 gm. of jelly.

It is the purpose of this communication to report some data collected on the ascorbic acid content of the West Indian cherry, as well as on

other chemical characteristics of fruit samples obtained from representative trees belonging to the experimental plantation maintained at the Agricultural Experiment Station of the University of Puerto Rico, at Río Piedras, Puerto Rico.

METHOD OF ANALYSIS

The fruits were collected early in the morning. They were weighed and measured, and the juice expressed and its chemical analysis determined during the following 24 hours.

Total Solids: The residue left after evaporating the juice at 100°C. (212°F.) for 24 hours.

pH: The pH of the juice was determined electrometrically with a glass electrode.

Total Acidity: Ten ml. of juice were titrated with 0.1 N NaOH and phenolphthalein as indicator. The result was expressed on the basis of 100 ml. of juice.

Reducing Sugars: The reducing sugars were determined using Benedict's reagent. Because ascorbic acid will also reduce this solution, a correction was applied; values reported are thus corrected for the ascorbic acid present.

Ascorbic Acid: Ascorbic acid was determined by the method of Bessey and King (2,6-dichlorophenol titration, 1933). The juice was initially diluted with a 2.5 per cent solution of orthophosphoric acid, but subsequent dilutions were made using nine per cent acetic acid.

RESULTS

The ripe fruit was usually larger than the partly ripe, average diameter of each being 2.2 and 1.7 cm., respectively. The weight of the ripe fruit was also greater than that of the green: 4.7 and 3.1 gm., respectively.

The amount of juice obtained, from the partly ripe fruit, by hand expression in a piece of cloth, averaged 55.3 per cent (maximum, 77.9 per cent, minimum, 41.0 per cent); while that obtained from the ripe fruit averaged 69.6 per cent (maximum, 80.2 per cent, minimum, 61.3 per cent). The total amount of juice in the partly ripe fruit averaged 83.3 per cent (maximum, 95.5 per cent, minimum, 74.3 per cent); and in the ripe fruit, 83.4 per cent (maximum, 91.0 per cent, minimum, 78.5 per cent). These values were computed by adding to the expressed juice the amount of juice left as moisture in the residue. The West Indian cherry juice has a beautiful reddish-orange color and an agreeable acid taste.

Results of the chemical analysis are reported (Table 1). Total solids per 100 ml. of juice fluctuated between 4.57 and 7.21, and the pH between 3.1 and 3.3, irrespective of degree of ripeness. Total acidity, expressed as ml. of 0.1 N NaOH necessary to neutralize the acid in 100 ml. of juice, was much higher in the juice from the partly ripe fruit (average, 160.7 ml.) than in that from the completely ripe (average, 135.9 ml.).

The amount of ascorbic acid in the West Indian cherry samples obtained were, with one exception (Sample B-8, ripe), over 1,000 mg. per 100 ml. of juice. The sample which gave the highest value was A-21 (partly ripe, 2,360 mg. per 100 ml.; and ripe, 1,522 mg. per 100 ml.); the one with the lowest content was B-8 (partly ripe, 1,338 mg. per 100 ml.; and ripe, 686

mg. per 100 ml.). In all samples the content of ascorbic acid for the partly ripe fruit was higher than for the ripe, thus confirming the previous observations of Asenjo and Freire de Guzmán (1946). The ascorbic acid per 100 gm. of whole fruit is reported (last column, Table 1).

TABLE 1

Some Chemical Characteristics of the West Indian Cherry Juice

Description	Total solids in juice	Juice	Total acidity of juice, ml. of 0.1 N NaOH per 100 ml. of juice	Reducing sugars in juice as glucose	Ascorbic acid in juice	Ascorbic acid in fruit
	gm./100 ml.	*pH*	*ml.*	*gm./100 ml.*	*mg./100 ml.*	*mg./100 gm.*
A-14—partly ripe	5.89	3.3	140.0	3.0	1,613	1,430
A-14—ripe	7.21	3.3	112.4	5.4	1,161	998
A-21—partly ripe	6.60	3.1	208.4	3.7	2,360	1,916
A-21—ripe	7.08	3.2	173.6	5.3	1,522	1,246
B-6—partly ripe	5.84	3.2	156.2	3.0	1,920	1,426
B-6—ripe	5.98	3.3	149.2	3.8	1,456	1,148
B-7—partly ripe	5.13	3.2	133.8	2.8	1,380	1,135
B-7—ripe	4.57	3.2	110.2	3.0	1,051	956
B-8—partly ripe	5.86	3.2	133.8	3.2	1,338	1,277
B-8—ripe	4.66	3.3	81.6	3.6	686	577
B-15—partly ripe	5.45	3.2	192.0	3.5	1,677	1,299
B-15—ripe	5.62	3.1	188.5	3.7	1,325	1,040

OTHER VITAMINS

Determinations were made of the thiamine, riboflavin, and niacin contents of different samples of West Indian cherry juice and the following results obtained:

	No. samples	Min.	Max.	Av.
		µg./100 ml.	*µg./100 ml.*	*µg./100 ml.*
Thiamine (thiochrome method)	6	12	33	24
Riboflavin (microbiological)	5	62	90	73
Niacin (microbiological)	3	470	490	480

These values are within the range of those reported for the same vitamins in the majority of other fruit juices.

DISCUSSION

The West Indian cherry is one of the richest, if not the richest, vegetable source of ascorbic acid known to man. The high production of the fruit, estimated at about two to six pounds per tree per harvest, makes it an important natural source of the above-mentioned acid.

The ascorbic acid produced per tree during one harvest may be estimated, very conservatively, at about 13 gm. Under actual commercial cultivation such a yield could easily be doubled.

There is a possibility that crystalline ascorbic acid could be produced economically by processing the juice of the fruit. Using quite rudimen-

tary methods, Asenjo and Freire de Guzmán (1946) obtained 1.25 gm. of acid from 275 ml. of a sample of juice containing, by analysis, approximately four gm. of ascorbic acid. This figure represents a recovery of only 31 per cent, which could doubtlessly be greatly improved under factory conditions.

Juice from the West Indian cherry could also be used advantageously as an enriching agent. Small amounts added to fruit juices poor in ascorbic acid (one to nine proportion) would produce blends with an ascorbic acid content well over that of the average orange juice, and would prove a very convenient manner of fortifying foods for ascorbic acid without having to resort to use of the synthetic product.

SUMMARY

The ascorbic acid content and other chemical characteristics of six representative types of the West Indian cherry (*Malpighia punicifolia* L.) have been studied. Thiamine, riboflavin, and niacin contents of the juice are also reported.

Possible uses of this fruit are discussed.

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INHIBITORY ACTION OF CITRIC ACID ON TOMATO JUICE FLAT-SOUR ORGANISMS

D. I. MURDOCK

Research Department, Continental Can Company, Inc.
Chicago, Illinois

[Received for publication, November 4, 1949]

Experiments were conducted to determine the bacteriostatic effect of citric and malic acids on the germination of spores of flat-sour organisms capable of producing spoilage in tomato juice. It was found that citric acid was highly inhibitory while malic acid was not. A relationship exists between the hydrogen ion concentration and the percentage of citric acid required for inhibition.

In 1933 Berry described a new type of spoilage organism responsible for an off-flavor in commercially canned tomato juice. The organism, which he named *Bacillus thermoacidurans*, is a non-gas-forming, facultative anaerobe having the general characteristics of the "flat-sour" type but is tolerant of an acid environment. It is of considerable importance in the spoilage of canned tomato juice in which it produces "off-flavors." Control of this type of spoilage in tomato canneries has been discussed by Cameron (1935), Pearce and Ruyle (1938), and others. Outbreaks of flat-sour spoilage in canned tomato juice occur every year but usually are of minor importance. In 1947, however, flat-sour spoilage in tomato juice was responsible for serious economic losses for a number of tomato juice canneries in the eastern part of the United States. The causative organisms may or may not have the same physiological characteristics as *B. thermoacidurans*, but they all produce an abnormal flavor in canned tomato juice.

In our study several strains of soil-borne organisms were isolated which produced this type of spoilage under favorable conditions of growth. It was noticed that addition of tomato juice to the culture media appeared to have a bacteriostatic effect upon growth of these organisms under laboratory conditions. The inhibitory effect of tomato juice when added to culture media was also noted by Stern, Hegarty, and Williams (1942). The nature of the specific inhibitory substance in the juice was not known. It is known that the pH of tomato juice ranges on the average between 4.0 and 4.2, that citric acid is the principal acid, and that malic acid is found in only small amounts. The inhibitory effect of organic acids upon germination of bacteria has been reported in the literature. Pederson and Breed (1926) and Levine and Fellers (1940) found acetic acid to be effective against bacteria. Erickson and Fabian (1942) reported the preserving and germicidal power for bacteria, judged by the amount of acid, to be in the order of lactic > acetic > citric. In view of these facts experiments were conducted to determine the effects of the naturally occurring acids of tomato juice upon germination of spores of flat-sour organisms.

EXPERIMENTAL PROCEDURE

Three organisms capable of producing off-flavor in tomato juice were used as test organisms. They will be referred to as Strains No. 24, 710, and 5. No. 24 was isolated from the soil known to have produced some of

the tomatoes used for juice, in which a high incidence of "flat-sour" spoilage occurred. Strain No. 710 was isolated from a spoiled can of "flat-sour" tomato juice packed in the same section of the United States. Strain No. 5 was a spore suspension of a flat-sour organism received from the National Canners' Association. All three strains produced terminal to subterminal spores and were facultative anaerobes growing slowly in tomato juice, with the production of an off-flavor without formation of gas. Spore suspensions were prepared from Strains No. 24 and 710 by growing them on proteose peptone acid agar pH 5. No. 24 was incubated at 30°C.(86°F.) and No. 710 at both 37 and 55°C.(98.6 and 131°F.).

The various factors under investigation were studied by the use of a basal medium which eliminated the interfering constituents normally present in tomato juice. The medium was prepared in double strength, to allow for dilution, as follows:

Tryptone	20 gm.
Dextrose	10 gm.
Water	1,000 ml.

Prior to sterilization the pH of the medium was adjusted in 500-ml. lots using hydrochloric, citric, or malic acids, so that on later dilution the desired pH could be obtained; the pH was measured electrometrically with a Coleman pH meter. Six different hydrogen ion concentrations were considered, ranging from pH 4 to 5. In each case the amount of acid required to adjust the lot was recorded. In the experiments where various concentrations of sodium citrate were added, the additional amount of acid required to readjust the medium to the original pH was also noted. Sodium hydroxide was used in one test where various concentrations of malic acid were added to the medium. Each lot of medium was distributed in eight-ounce screw-cap bottles, 75 ml. per bottle, and sterilized by autoclaving 30 minutes at 121°C.(250°F.). A three per cent agar solution was distributed, 75 ml. per bottle, and also sterilized. The mixing of one bottle of agar and one bottle of liquid medium resulted in a medium of standard composition at the desired pH values.

Growth of the test organisms on the basal medium, adjusted to the desired pH level with hydrochloric acid, was compared with growth obtained on the media containing the various acids investigated. This was done by plating out the standardized spore suspensions of the three test organisms. Unless otherwise stated all plates were incubated five days; Organism No. 24 at 30°C.(86°F.) and Organisms No. 710 and 5 at 55°C. (131°F.). While these organisms are capable of growing over the entire range of temperature (30 to 55°C.), the optimum growth temperature for each organism was not ascertained. However, preliminary methods indicated Organism No. 24 grew more rapidly at the lower temperature, while the other two organisms preferred higher temperatures.

RESULTS AND DISCUSSION

The effect of pH alone is shown (Table 1). While the reduction in the pH reduced the number of spores germinating, complete inhibition of the test organisms did not occur until a pH of 4 was obtained. Where the

TABLE 1

Effect of pH on Growth of Flat-Sour-Producing Organisms

Basal Medium Acidified With Hydrochloric Acid

pH of medium	No. spores germinating per ml.		
	No. 24	No. 710	No. 5
5.0	5,800,000	520,000	160,000
4.8[1]	4,550,000	475,000	212,500
4.6	3,020,000	420,000	121,000
4.4	1,510,000	380,000	128,500
4.2	56,000	260	2,000
4.0	<10	<10	<10

[1] Indicates the average of two determinations.

sign < (less than) is used, it means that sterile plates were obtained at the dilution indicated.

The effect of citric acid or the citrate ion at several hydrogen ion concentrations is shown (Table 2). At pH 5 very little bacteriostatic effect was noted. At pH 4.8 a very noticeable inhibition begins and increases as the pH is lowered. Bacterial growth occurring on the basal medium adjusted to pH 4.4 with hydrochloric acid is shown (Fig. 1), and Fig. 2 shows the

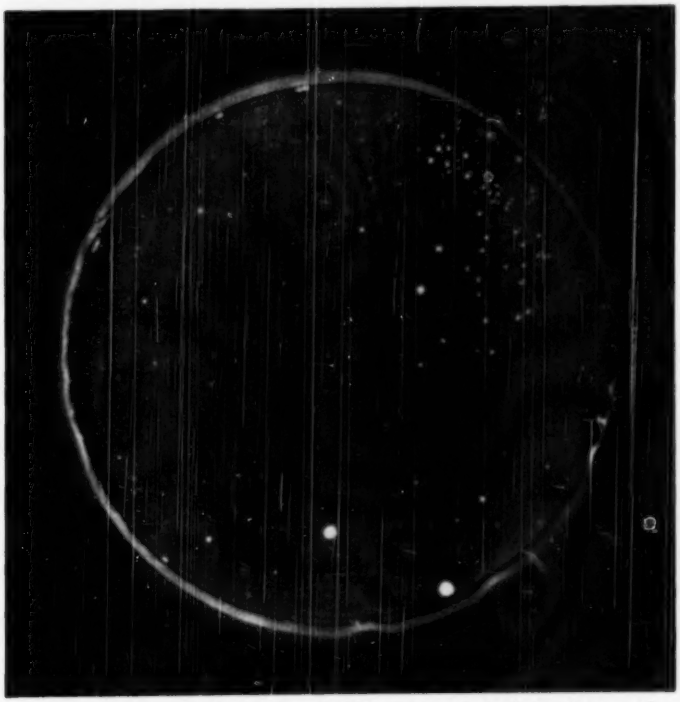

FIG. 1. Growth on basal medium adjusted to pH 4.4 with hydrochloric acid.

TABLE 2

Effect of Citric Acid on Growth of Flat-Sour-Producing Organisms Isolated From Tomato Juice[1]

Test No.	Na Citrate added to medium	Medium acidified with	(Anhydrous) Citric acid in final media	No. spores germinating per ml.		
				No. 24	No. 710	No. 5
			Basal Medium Adjusted to pH 5			
	pct.		*pct.*			
1	0	HCl	0.046	15,150,000	450,000	160,000
	0.2	Citric	0.240	3,230,000	82,000	160,000
	0.4	Citric	0.429	3,090,000	680	98,000
	0.6	Citric	0.613	5,300,000	18,000	120,500
	0.8	Citric	0.722	5,220,000	50,000	100,000
2	0	HCl	0	5,800,000	520,000	160,000
	0	Citric	0.046	4,600,000	340,000	130,000
	0.2	Citric	0.240	4,500,000	128,000	130,000
	0.4	Citric	0.429	3,500,000	1,800	73,000
	0.6	Citric	0.613	2,230,000	1,100	55,000
	0.8	Citric	0.722	3,500,000	<100	32,000
	1.0	Citric	0.904	2,700,000	< 10	28,000
			Basal Medium Adjusted to pH 4.8			
3	0	HCl	0	4,300,000	430,000	225,000
	0.2	Citric	0.256	<10	< 10	< 10
	0.4	Citric	0.442	<10	< 10	710
	0.6	Citric	0.626	<10	< 10	830
	0.8	Citric	0.804	<10	< 10	< 10
	1.0	Citric	0.978	<10	< 10	< 10
			Basal Medium Adjusted to pH 4.6			
4	0	HCl	0	3,020,000	420,000	121,000
	0.2	Citric	0.313	<10	< 10	< 10
5	0	HCl	0	810,000	640,000	221,000
	0.2	HCl	0.13	65,000	< 10	<300
	0.4	HCl	0.26	63,000	< 10	< 10
	0.6	HCl	0.39	14,600	< 10	< 10
	0.8	HCl	0.52	<10	< 10	< 10
			Basal Medium Adjusted to pH 4.4			
6	0	HCl	0	1,510,000	380,000	128,500
	0	Citric	0.120	<10	< 10	< 10
7	0	HCl	0	130,000	210,000	5,600
	0	Citric	0.120	<10	< 10	< 10
			Basal Medium Adjusted to pH 4.2			
8	0	HCl	0	56,000	260	2,000
	0	Citric	0.140	<10	< 10	< 10
			Basal Medium Adjusted to pH 4.0			
9	0	HCl	0	<10	< 10	< 10
	0	Citric	0.176	<10	< 10	< 10

[1] All plates were incubated five days; No. 24 at 30°C., No. 710 and No. 5 at 55°C.

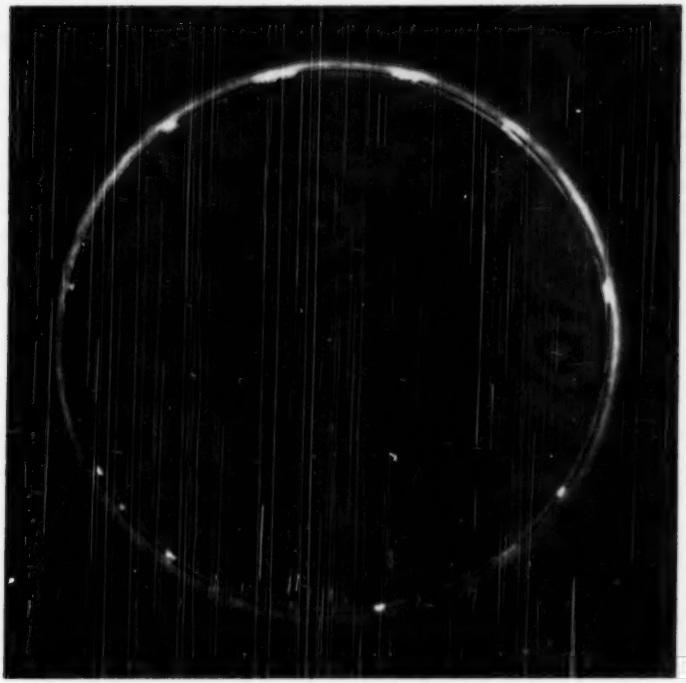

FIG. 2. Absence of growth on basal medium adjusted to pH 4.4 with citric acid.

absence of bacterial growth when the same medium was adjusted with citric acid to this pH.

It is apparent from the results presented (Tables 1 and 2) that the chloride ion has little or no effect upon spores of the test organisms. On the other hand, the citric acid or the citrate ion has a very noticeable effect. At pH 5 a concentration of 0.904 per cent citric acid was only slightly bacteriostatic to the spores of the three test organisms, while at pH 4.8, 0.256 per cent citric acid was markedly inhibitory. At pH 4.6, 0.13 per cent citric acid was inhibitory. Critical inspection of these tables shows a few minor discrepancies, but the overall trend indicates the relationship of citric acid to inhibition.

Results presented (Table 3) indicate 0.21 per cent malic acid at pH 4.4 is inhibitory. However, according to Winton and Winton (1935), malic acid as free acid never is in excess of 0.05 per cent in tomatoes. Therefore, the malic acid in the medium at pH 4.4 is approximately four times the amount normally found.

An analysis for citric acid of 63 different samples of canned tomato juice from Indiana and Eastern United States, by a volumetric pentabromacetone procedure as outlined by Pucher, Vickery, and Leavenworth

(1934), indicated the samples ranged from 0.216 to 0.465 per cent citric acid, with an average of 0.347 per cent. The pH averaged 4.1 (ranging from 3.95 to 4.25).

From the data obtained with laboratory media one would conclude that bacteria responsible for flat-sour spoilage in tomato juice should not grow in the juice in the citric acid ranges found. This discrepancy in results may be due to the complex composition of tomato juice in comparison to the laboratory media used for the study. Also, it is possible that other factors are present in tomato juice which decrease the bacteriostatic effect of citric acid found on artificial media, or stimulate the germination of spores not germinating in the laboratory media. However, when the flat-sour tomato juice problem has been fully explored it may indicate that citric acid is an important factor in regard to germination of spores surviving processing.

TABLE 3

Effect of Malic Acid on Growth of Flat-Sour-Producing Organisms Isolated From Tomato Juice[1]

Basal Medium Adjusted to pH 4.8

Test No.	Malic acid in final medium	pH of media	Medium adjusted to pH with	No. spores germinating per ml.		
				No. 24	No. 710	No. 5
	pct.					
10	0	4.8	HCl	4,500,000	420,000	180,000
	0.01		HCl	3,400,000	320,000	170,000
	0.05		NaOH	3,300,000	360,000	200,000
	0.1		NaOH	4,100,000	360,000	210,000
	0.2		NaOH	3,600,000	340,000	190,000
11	0	4.8	HCl	320,000	740,000	8,300
	0.114		.....	300,000	550,000	6,300
	0	4.6	HCl	350,000	490,000	7,600
	0.160		.....	190,000	270,000	4,300
	0	4.4	HCl	130,000	210,000	5,600
	0.207		.....	7,500	<10	<10

[1] All plates were incubated five days; No. 24 at 30°C., No. 710 and No. 5 at 55°C.

It was noticed in the course of the study that a chance contamination by a mold allowed growth on a plate seeded with flat-sour spores in a medium adjusted to pH 4.8 with citric acid. Application of brom cresol purple indicator to the plate indicated the pH had been increased to the low acid or neutral range where growth was apparent. Obviously, the acid had either been utilized or neutralized by the mold in its metabolic process.

SUMMARY

A series of experiments was conducted to determine the bacteriostatic effect of citric and malic acids on the germination of spores of flat-sour-producing organisms. The data obtained indicate that citric acid is highly inhibitory to the strains of flat-sour organisms studied. Malic acid in sufficient concentrations has an inhibitory effect; however, it is present in tomato juice, according to the literature, in only minute amounts. Lowering the pH with hydrochloric acid results in a decrease in the number of spores germinating, but complete bacteriostasis did not occur even at pH

4.2. The relationship between the hydrogen ion concentration of the medium and amount of citric acid required for inhibition has been shown.

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance of J. Dahl in making the analysis for citric acid, of J. M. Boyd and C. F. Schmidt for their technical assistance, and of V. S. Troy for reviewing the paper.

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THERMAL DESTRUCTION AND STABILITY OF PEROXIDASE IN ACID FOODS[1,2]

EDWARD A. NEBESKY, WILLIAM B. ESSELEN, JR., A. M. KAPLAN, AND CARL R. FELLERS

Department of Food Technology, University of Massachusetts, Amherst, Massachusetts

[Received for publication, November 7, 1949]

The present investigation is a continuation of that reported by Kaplan, Esselen, and Fellers (1949), expanded to determine the correlation between a calculated thermal process, based on heat penetration and thermal destruction time data, with the actual process time required for the destruction of peroxidase in canned acid foods. The thermal destruction times of peroxidase, as determined by use of various substrates to detect the presence or absence of enzyme activity, have been considered. Data on the effect of concentration on the thermal stability of peroxidase have also been obtained.

REVIEW OF LITERATURE

In spite of the recognition that viable enzymes may play a role in the deterioration of certain heat-processed foods, little work has been reported in the literature on the thermal destruction characteristics of such enzymes as they might apply to canned acid foods.

Cruess, Quin, and Mrak (1932) studied the browning of canning peaches and the destruction temperature of peach oxidase. Tressler (1947) stated that apple, pear, plum, peach, and apricot juices will turn brown owing to action of peroxidase. Overholser and Cruess (1923), Joslyn and Marsh (1934), Hussein and Cruess (1940), Strachan (1942), Joslyn and Sedky (1940), Parks (1940), Kertesz (1939), Woodroof and Atkinson (1944), and others have made observations on the destruction temperature of certain enzyme systems of various fruit.

The problems involved in the destruction of enzyme activity by heat have been summarized by Balls (1942, 1944), who stressed the difficulty of complete destruction, possibility that the products of decomposition by heat might exert slight catalytic action, and possible regeneration of activity after destruction. Such regeneration is well recognized and has been known to occur with proteolytic enzymes and peroxidase as reported by Hand (1933), Schwimmer (1944), Reddi, Esselen, and Fellers (1949), and others.

Although data have been reported concerning the heat inactivation of enzyme systems of acid foods, relatively little attention has been given to the time-temperature relationships for destruction of enzymes in the canned or processed product. Ball (1943, 1948) recognized that such relationships might be similar to those affecting the thermal destruction of microorganisms. Parks (1940) and Joslyn and Sedky (1940) reported work on the heat process requirements of citrus juices necessary to de-

[1] Contribution No. 739, Massachusetts Agricultural Experiment Station.

[2] Presented before the Division of Agricultural and Food Chemistry of the American Chemical Society, Atlantic City, New Jersey, September 19, 1949.

stroy pectinase enzymes, which caused a curd formation in the canned product. Sanders and Sager (1948) described a laboratory pasteurizer used for controlling the time-temperature relationships, with a high degree of precision, in studies on the phosphatase inactivation of milk and dairy products. They obtained a straight-line destruction curve for phosphatase in fluid dairy products, when the logarithms of the heating times were plotted against the corresponding temperatures.

Kaplan, Esselen, and Fellers (1949) studied the effect of processing conditions of time and temperature upon the peroxidase and other enzyme systems of canned acid foods such as fruits and pickles. They obtained good agreement between calculated processes for the destruction of peroxidase, based on thermal destruction and heat penetration data, and actual processed products in the case of puréed foods. Some discrepancies were observed in the case of products such as pickles packed as whole pieces. They attributed these discrepancies to the effect of penetration of sugar or acids into the pieces of product during processing.

EXPERIMENTAL PROCEDURE

Methods: Thermal destruction times for peroxidase in different products, and heat penetration data for the canned products, were determined as previously described by Kaplan, Esselen, and Fellers (1949). Data so obtained were treated by the General Method of Process Time Determination, Bigelow, Bohart, Richardson, and Ball (1920) and Ball (1928), with the necessary adaptations for peroxidase, rather than microorganisms as described by Kaplan, Esselen, and Fellers (1949). Heat penetration data were obtained from four points in a jar per processing run by inserting copper-constantan thermocouples into the center of three different pieces of the product located in the lower portion of the jar. The fourth thermocouple was located in the syrup or brine at the predetermined point of slowest heating. Pint jars were used for fruits and quart jars for pickles. Twelve processing runs were made with each product and composite heating and cooling curves, which represented the slowest heating and fastest cooling, were made and used for calculation of the process time required for destruction of peroxidase. Processing was done in a water bath, with an automatic temperature controller.

Thermal destruction times for peroxidase in the various products were determined at four or more different temperatures between 71.1°C. (160°F.) and 100°C. (212°F.) in Pyrex thermal destruction tubes heated in a constant temperature oil bath. Extracts of the foods were prepared by blending 25 gm. of the food with 44 ml. of distilled water, brine, or syrup in a Waring Blendor. Two ml. of the extract were placed in each thermal destruction time tube which was sealed in an oxygen flame. The tubes were placed in the oil bath and at the end of the heating period were removed and immediately immersed in ice water to insure rapid cooling. The tubes were then opened and their contents mixed together to obtain a representative sample (composite of these tubes). The sample was then tested for peroxidase activity by adding one ml. to nine ml. of distilled water. One ml. of 0.3 per cent hydrogen peroxide and one ml. of a one per cent ethanol solution of guaiacol were added. Development of the

characteristic red-brown color was taken as indicating presence of active peroxidase. A lag correction factor of 1.5 minutes was employed for the heating of the thermal destruction tubes, as previously determined.

Terms employed in describing the thermal destruction characteristics of peroxidase may be defined as follows:

F_{212}—Number of minutes required to destroy the peroxidase, in any specific medium, at 100°C.(212°F.).

F_{190}—Number of minutes required to destroy the peroxidase, in any specific medium, at 87°C.(190°F.).

z—Slope of the thermal destruction time curve expressed as degrees Fahrenheit. This is the interval in temperature required for the curve to pass through one log cycle on semi-logarithmic paper.

Thermal Destruction of Peroxidase: Thermal destruction times for peroxidase in sliced apples, apple juice, peaches (halves), pears (halves), and fresh and salt stock pickles were determined at from four to six different temperatures ranging from 71.1 to 100°C.(160 to 212°F.). In the case of apples and pears the effect of various concentrations of sugar (sucrose) was determined, and the effect of vinegar ascertained in the case of pickles. Thermal destruction time curves constructed from the above data were found to follow a straight line and their F_{190} or F_{212} and z values are shown (Table 1). Typical curves are shown (Fig. 1).

TABLE 1

Comparison of Calculated Process Time With Observed Process for Destruction of Peroxidase

Product	F_{190}	F_{212}	z	RT	Calculated process time	Observed process time	
						Survival	Destruction
	min.	*min.*		°*F.*	*min.*	*min.*	*min.*
Apple (plain)	....	0.70	33.5	212	15.5	10	15
Apple (20% syrup)	....	0.72	28.0	212	15.5	10	15
Apple (40% syrup)	....	0.75	27.0	212	20.5	15	20
Apple cider	3.2	....	32.5	190	24.5	20	25
Peach (plain)	0.41	....	31.0	212	31.0	30	35
Pear (plain)	4.0	....	32.0	190	38.0	35	40
Pear (25% syrup)	4.2	....	27.5	190	44.5	40	45
Pear (50% syrup)	4.5	....	25.5	190	48.5	40	45
Pickle (fresh)	....	1.7	22.0	186	45.5	35	40
Pickle (salt stock plain water pack)	....	4.5	33.0	190	36.5	35	40
Pickle (salt stock 5.0% vinegar pack)	....	0.48	33.0	190	21.0	15	20

It may be seen (Table 1) that addition of sugar tended to increase the thermal stability of peroxidase in apples and pears. Its stability was markedly reduced in pickles in the presence of vinegar.

In order to check the validity of the above thermal destruction times for peroxidase, experimental lots of each product were canned in jars and processed for various periods, at five-minute intervals in a water bath at the temperatures indicated (Table 1). Rates of heat penetration into the pieces of product, as well as the syrup or brine, were measured during heating and cooling, as previously described. Tests for presence of active

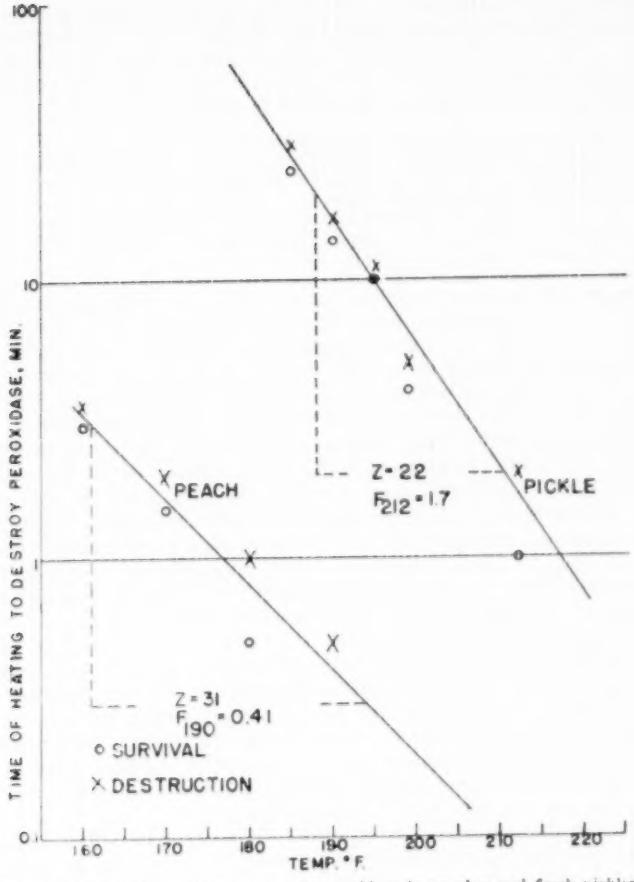

FIG. 1. Thermal destruction curves of peroxidase in peaches and fresh pickles.

peroxidase were made on the canned products, in order to determine the approximate process time necessary to inactivate the enzyme. Heat penetration and thermal destruction data were treated by the General Method to calculate a theoretical process time for destruction of the enzyme. Calculated and actual process times required for the destruction of peroxidase are shown (Table 1). Reasonably good agreement was obtained between calculated and process times actually required for products consisting of separate pieces in a brine or syrup. These findings confirm those obtained by Kaplan, Esselen, and Fellers (1949) on puréed fruits, in showing a good agreement between calculated and actual process times required for destruction of peroxidase.

Effect of Test Substrate on Thermal Stability: Preliminary data had indicated that the apparent thermal destruction times for peroxidase in certain products varied according to the substrate used in testing for presence of the active enzyme. Accordingly, enzyme assays for peroxidase were carried out, using several substrates as follows:

Hydroquinone (one per cent in distilled water)
Guaiacol [one per cent in ethanol (95 per cent)]
Pyrogallol [one per cent in ethanol (95 per cent)]
o-Phenylenediamine dihydrochloride
(one per cent in distilled water, pH adjusted to 4.5 with NaOH)
Benzidine [one per cent in ethanol (95 per cent)]
Catechol [0.5 per cent in ethanol (95 per cent)]

Thermal destruction times were determined for peroxidase as measured with the above substrates in apples, apple juice, peaches, pears, and pickles, at four temperatures and results summarized (Table 2). Tests for enzyme activity were performed by adding one ml. of the heated product, from TDT tubes, to nine ml. distilled water. One ml. of 0.3 per cent hydrogen peroxide and one ml. of the respective substrate were then added.

Results: In determining enzyme activity using the above-mentioned substrates with TDT studies and with processed packs, peroxidase activity differed with the substrate in some cases. For example, tests with apple slices and apple cider using guaiacol, pyrogallol, or catechol as the substrate, produced results which were identical and showed these enzymes to be of higher thermal resistance than hydroquinone, benzidine, and o-phenylenediamine dihydrochloride. Similar variations appeared among the various substrates studied with the other products concerned in this investigation (illustrated Table 2).

From the above studies guaiacol peroxidase was found to have as great or greater thermal resistance than peroxidase, measured with other substrates. Hence, guaiacol was selected as the preferable substrate to use in measuring peroxidase activity and destruction under these conditions. The guaiacol substrate also produced a much more distinct difference in color, which would be very easily recognized, and thereby tended towards greater accuracy in carrying out the enzyme assay.

Effect of Concentration of Peroxidase: Concentration of peroxidase in a given product may be subject to variation as influenced by maturity, variety, rate of respiration, and previous storage conditions. Hence, studies were carried out to determine the effect of the enzyme concentration on its thermal destruction time. This necessitated use of a suitable technique for purification and concentration of the peroxidase in a given food product. A modification of the method of Hussein and Cruess (1940), for preparation of a purified peroxidase extract from grapes, was applied to apples and cucumbers as follows:

Four hundred gm. of peeled apples or unpeeled cucumbers were added to 400 ml. of acetone and mixed in a Waring Blendor three minutes, followed by grinding in a porcelain mortar with clean sand until the tissues were well divided. The macerated tissue was then washed with 400-ml. amounts of acetone 12 times. Acetone usually removes oxidizable color bases and acts as a dehydrating agent, as well as removing tannins and

TABLE 2

Effect of Test Substrates on Thermal Stability of Peroxidase From Various Foods

Substrate	Survival and destruction time									
	160°F.		175°F.		190°F.		212°F.		F_{250}	z
	Sur.	Dest.	Sur.	Dest.	Sur.	Dest.	Sur.	Dest.		
Apple Slices	min.		min.		min.		min.		min.	min.
Guaiacol	20	25	7	9	3	4	0.5	1.0	.70	33.5
Pyrogallol	20	25	7	9	3	4	0.5	1.0	.70	....
Catechol	20	25	7	9	3	4	0.5	1.0	.70	33.5
Hydroquinone	10	15	4	6	2	3	....	0.5	.48	34.5
Benzidine	....	....	....	....	....	....	....	....	....	....
o-Phenylenediamine	....	....	....	....	....	....	....	....	....	....
Apple Juice										
Guaiacol	20	25	7	9	3	4	0.5	10	.61	32.5
Pyrogallol	20	25	7	9	3	4	0.5	10	.61	32.5
Catechol	20	25	7	9	3	4	0.5	10	.61	32.5
Hydroquinone	10	15	4	6	2	3	....	0.5	.48	34.5
Benzidine	....	....	....	....	....	....	....	....	....	....
o-Phenylenediamine	....	....	....	....	....	....	....	....	....	....
Peaches										
	160°F.		170°F.		180°F.		210°F.		F_{200}	z
Guaiacol	3.0	3.5	1.5	2.0	0.5	1.0	....	0.5	.40	31.0
Pyrogallol	....	....	....	....	....	....	....	....	....	....
Catechol	....	....	....	....	....	....	....	....	....	....
Hydroquinone	....	....	....	....	....	....	....	....	....	....
Benzidine	3.0	3.5	1.5	2.0	0.5	1.0	....	0.5	.40	31.0
o-Phenylenediamine	....	....	....	....	....	....	....	....	....	....
Pears										
	160°F.		175°F.		180°F.		212°F.		F_{250}	z
Guaiacol	30	35	16	18	8	10	0.5	1.0	.86	32.0
Pyrogallol	30	35	16	18	8	10	0.5	1.0	.86	32.0
Catechol	30	35	16	18	8	10	0.5	1.0	.86	32.0
Hydroquinone	30	35	16	18	8	10	0.5	1.0	.86	32.0
Benzidine	30	35	16	18	8	10	0.5	1.0	.86	32.0
o-Phenylenediamine	30	35	16	18	8	10	0.5	1.0	.86	32.0
Freshened Salt Stock Pickles										
	160°F.		180°F.		190°F.		212°F.		F_{250}	z
Guaiacol	35	40	8	10	4	6	0.5	1.0	.96	33.0
Pyrogallol	....	....	....	....	....	....	....	....	....	....
Catechol	15	20	2	4	....	0.5	....	....	.08	19.0
Hydroquinone	....	....	....	....	....	....	....	....	....	....
Benzidine	15	20	2	4	....	0.5	....	....	0.10	24.0
o-Phenylenediamine	....	....	....	....	....	....	....	....	....	....

.... = No activity.

sugars which, according to Onslow (1921), are enzyme inhibitors. Washing with acetone was repeated until the solvent completely lost its yellow-green color. The acetone was then filtered off and 400 ml. of a phosphate buffer, pH 5.0, was added to the residue and allowed to stand two hours, to permit extraction of the enzyme. The extract was then filtered in Buchner funnel, through a layer of neutral asbestos. The enzyme in the filtrate was precipitated by adding an amount of 95 per cent ethanol equal to three times the volume of the filtrate. The precipitate found was white and flocculent. It was centrifuged and immediately resuspended in 50 ml. of phosphate buffer. The enzyme was then reprecipitated with ethanol, centrifuged, and immediately suspended in 50 ml. of phosphate buffer. The resulting peroxidase extract was clear and colorless.

The concentration of peroxidase activity of the original extract, and dilutions of it in phosphate buffer was determined colorimetrically, as described by Morris, Weast, and Lineweaver (1946), using an Evelyn Photoelectric colorimeter (Filter No. 420) and guaiacol as the substrate. The original enzyme preparation was found to be 50 times more concentrated, on a basis of enzyme activity, than the peroxidase concentration in the original fruit.

The effect of the concentration of peroxidase on its thermal stability is shown (Table 3). It may be seen that an increase in the concentration of peroxidase increased its resistance to heat inactivation.

Variation in Thermal Stability of Peroxidase: Studies were undertaken to observe the peroxidase activity of a number of different lots of fresh packs of Kosher style dill pickles, processed under commercial conditions in various pickle-packing plants during the fresh pack season, and also on similar packs put up in this laboratory. Experimental packs of washed and unwashed pickles, and packs with mustard oil added at the rate of 20 p.p.m. were studied. Processing runs were made in a water bath at 82.2°C.(180°F.) and two jars removed at five-minute intervals up to 40 minutes, and assayed for peroxidase. Samples taken during the various processing runs showed that peroxidase was not destroyed in jars processed for 40 minutes at 82.2°C.(180°F.) in any of the lots studied. However, samples from the various lots did vary in intensity of color produced in the enzyme test, from a very light brown to a dark red-brown, thereby indicating that peroxidase activity was fairly light in the former case, while in the latter the peroxidase was very strong.

Addition of mustard oil at the rate of 20 p.p.m. was found to have no effect on inhibiting peroxidase activity.

Enzyme tests made to determine the thermal resistance of peroxidase in fresh cucumbers, obtained from similar sources on different days as above, revealed that thermal resistance of peroxidase varied between lots, which is in agreement with the above studies. As illustrated (Table 4, for example), one lot showed peroxidase to be destroyed between 40 and 45 minutes at 87.8°C.(190°F.), while on another lot peroxidase was destroyed between 150 and 200 minutes at 87.8°C.(190°F.). Similar variations appeared among the other lots studied at 87.8 and 93.6°C.(190 and 200°F.). Only slight variations appeared at 100°C.(212°F.), as this higher temperature decreased the margin of difference in time necessary to destroy the enzyme.

TABLE 3

Thermal Resistance of Various Concentrated Peroxidase Extracts From Apples and Cucumbers

Concentration	Survival and destruction time								F_{212}	z
	160°F.		175°F.		190°		212°F.			
	Sur.	Dest.	Sur.	Dest.	Sur.	Dest.	Sur.	Dest.		
Apples										
	min.		min.		min.		min.		min.	min.
Concentration equivalent to concentration in original fruit	15	20	4	6	1.0	1.5	0.0	0.5	0.19	26.2
Concentration 25 times greater than original	35	40	12	15	4.0	6.0	0.5	1.0	0.91	32.8
Concentration 50 times greater than original	70	75	40	45	21	24	8	10	9.00	55.4
Cucumbers										
	160°F.		175°F.		190°		210°F.		F_{212}	z
Concentration equivalent to concentration in original raw material	25	30	8	10	3	4	0.5	1.0	0.71	33
Concentration 50 times greater than original	90	100	50	55	30	35	10	12	14.20	64

TABLE 4

Variation in Thermal Stability of Peroxidase in Fresh Cucumbers

Sample	Survival and destruction time					
	190°F.		200°F.		212°F.	
	Sur.	Dest.	Sur.	Dest.	Sur.	Dest.
	min.		*min.*		*min.*	
Lot 1	80	90	15	20	1.5	2.0
Lot 2	150	200	25	30	2.0	2.5
Lot 3	80	90	10	15	1.5	2.0
Lot 4	40	45	7	9	1.5	2.0
Lot 5	90	100	10	15	1.5	2.0
Lot 6	80	90	9	11	1.5	2.0
Lot 7	70	80	9	11	1.5	2.0
Lot 8	45	50	7	9	1.5	2.0
Lot 9	80	90	10	15	1.5	2.0

The above observations are indicative that considerable variation in thermal stability of peroxidase occurs within different lots of the same product, and that this difference may be attributed to the peroxidase concentration in the product.

Stability of Peroxidase During Storage of Processed Packs: Samples from various packs of Kosher style dill pickles processed for time periods of five, 10, 15, 20, 25, 30, 35, and 40 minutes, respectively, at 85°C. (186°F.), and stored at room temperature for a period of approximately one year, were assayed for peroxidase activity. Results showed the enzyme to be present in packs processed 35 minutes and destroyed in packs processed 40 minutes, which was in agreement with results obtained on similar samples assayed immediately after completion of the process. The evidence presented is indicative that peroxidase which was not inactivated in fresh cucumber pickles during processing was not destroyed during storage for one year.

DISCUSSION OF RESULTS

Data have been obtained concerning the thermal destruction and stability of peroxidase in such acid foods as whole fresh and salt stock pickles, peach and pear halves, and apple slices. Evaluation of calculated process times through the processing of experimental packs showed the calculated process time to be in close agreement with the observed process time (illustrated Table 1). These findings confirm those obtained by Kaplan, Esselen, and Fellers (1949) on puréed fruits.

In addition to a consideration of the thermal destruction characteristics of peroxidase *per se*, the effect was investigated of such additives as sugar, salt, and vinegar upon thermal destruction characteristics, since these substances are used in the canning and packing of various acid foods. Because these substances may influence enzyme inactivation when added to solid food products, the extent of their penetration into the food during preparation and processing must be considered. Sugar penetration into fruit products, and salt and vinegar penetration into pickle products, were studied. The soluble solids contents of the various packs before and after the process were determined by means of a refractometer. An increase in sugar concentrations in thermal destruction time studies resulted in a decrease in z value, and an increase in F_{212} or F_{190} value, indicating

that addition of sugar increased resistance of the apple and pear peroxidase to inactivation by heat.

Vinegar content of the various packs before and after the process was determined by measuring the total acidity. Studies of thermal destruction time revealed that increasing vinegar concentrations produced no change in the z value, but a decrease in the F_{212} value. This indicates that the addition of a 2.5 and five per cent vinegar solution markedly decreases resistance of the pickle peroxidase to inactivation by heat. Addition of small amounts of salt (two per cent) had no effect on the thermal stability of pickle peroxidase. Calculated process times for the above-mentioned packs are in close agreement with observed process times (as illustrated Table 1).

On the basis of the work carried out in this study it is felt that further investigations are indicated on other solid food products, as well as fruit juices, to determine the exact time-temperature relationships for destruction of peroxidase in these types of foods. Possibility that peroxidase may play a role in deterioration of canned foods has been indicated. In the case of juices, the primary purpose of the pasteurization process is to kill all microorganisms which may develop in the product and thus change its character; also, to inactivate enzymes which may cause deterioration. As pointed out by Pederson and Beavens (1940), the temperature necessary in kettle pasteurization, for complete killing of microorganisms in grape juice, is comparatively low, that is, 65.6°C.(150°F.). This, incidentally, is apparently below the temperature required for the inactivation of all enzymes, since definite enzymatic changes have been noted in juices pasteurized at 65.6 to 68.3°C.(150 to 155°F.), even though all microorganisms had been destroyed. Therefore, the importance of enzyme systems in such products should be taken into consideration.

With development of further information it is possible that destruction of peroxidase in canned acid foods may prove useful in some cases as a measurement of adequate processing. Under such conditions it would be necessary to be assured that the peroxidase was more heat-resistant than potential spoilage microorganisms which might be encountered. However, our findings to date indicate that the concentration of peroxidase activity in the raw product may be subject to considerable variation, and that a better understanding of this phase of the problem is necessary before considering the practical use of peroxidase inactivation as a test for adequate processing in canned fruits and pickles.

SUMMARY AND CONCLUSIONS

Studies to determine the effect of time and temperature relationships during processing, on the peroxidase of canned acid foods, have been continued. Although the destruction of microorganisms in processed foods has been emphasized, attention should also be given in the destruction of the enzymes, since they also play an important role in food preservation. Calculated and observed process times for destruction of peroxidase showed good agreement.

Guaiacol peroxidase was found to have a greater thermal resistance than peroxidase measured with other substrates such as o-phenylenediamine, catechol, benzidine, hydroquinone, and pyrogallol. Hence, guaiacol

was selected as the preferable substrate to use in measuring peroxidase activity and destruction under these conditions. The significance of these observations is discussed.

Addition of sugar or vinegar to the products studied increased and decreased, respectively, the thermal stability of peroxidase, while the addition of small amounts of salt (NaCl) had no effect. Increasing the concentration of peroxidase increased its thermal stability. Peroxidase which was not inactivated in fresh cucumber pickles during processing was not destroyed during storage for one year.

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VIABILITY OF FOOD-POISONING *STAPHYLOCOCCI* AND *SALMONELLAE* IN SALAD DRESSING AND MAYONNAISE [1]

MARY CATHERINE WETHINGTON AND F. W. FABIAN
Department of Bacteriology and Public Health, Michigan State College, East Lansing, Michigan

[Received for publication, November 14, 1949]

Possibility of a food being a carrier of disease-producing agents is an important consideration from the standpoint of manufacturing and consumption. Salad dressing and mayonnaise both contain eggs, which have been responsible for epidemics of food poisoning caused by various species of *Salmonellae*. Jordan [see Newberry (1943)] and his colleagues in the United States and Kauffmann [see Newberry (1943)] in Germany have demonstrated that certain recognized types of *Salmonellae* are the common cause of food-poisoning outbreaks. More recently Edwards and Brunner (1943) isolated *Salomonella pullorum* from a patient suffering from gastro-enteritis contracted from eggs. Mitchell, Garlock, and Broh-Kohn (1946) reported *S. pullorum* to be responsible for an outbreak in which 423 persons required medical treatment. Topley and Wilson (1936) stated that food poisoning due to *Salmonella enteritidis* and *Salmonella typhimurium* has often been traced to duck eggs. Judefind (1947) reported a case of *S. pullorum* food poisoning due to eggs which were insufficiently cooked.

Dack, Fordon, and Woolper (1936) and others showed that certain strains of *Staphylococci* produced toxic substances responsible for a considerable number of food-poisoning outbreaks.

Many foods contaminated with food-poisoning bacteria cause gastro-intestinal symptoms. Meat and meat products have proved to be one of the commonest sources of infection, while mixed foods such as hashes and meat pies are involved in many cases.

The enterotoxin of food-poisoning *Staphylococci* is more commonly found in such foods as custards, chocolate eclairs, and cream sauces. *Salmonellae* are often isolated from cases of food poisoning due to the same type of food products, also to meat and eggs. It is evident these two groups of organisms, the *Staphylococci* and the *Salmonellae*, find to their liking the same kind of food.

A study was made to determine the possibility of salad dressing and mayonnaise causing food poisoning, since they contain eggs, and since there have been no reported cases of food poisoning traced to them.

I. VIABILITY OF FOOD-POISONING *STAPHYLOCOCCI* AND *SALMONELLAE* IN COMMERCIAL SALAD DRESSING AND MAYONNAISE

Three strains of food-poisoning *Staphylococci*, numbered 172, 178, and 196, were used in the study. The cultures were obtained from Dr. G. M. Dack, Food Research Institute, University of Chicago, and had been isolated previously from chipped beef and two different samples of ham.

[1] Journal Article No. 1,094 (n.s.).

Dack has proved the toxicity of these strains several times by the use of monkeys. In addition to the above strains of food-poisoning *Staphylococci, S. typhimurium, S. enteritidis, S. pullorum, Salmonella paratyphi, Salmonella schottmuelleri,* and *Salmonella choleraesuis* were also used in the study.

One ml. of a 24-hour culture of each of the above cultures of bacteria was added to 25-gram samples of salad dressing and mayonnaise made under commercial conditions. To simulate conditions that occur in commercial products, the samples were allowed to stand at room temperature, then cultured at various time intervals. Samples were also incubated at 37°C.(99°F.), the optimum temperature for the bacteria, and cultured at various time intervals. Difco's MacConkey medium was used as the plating medium for the *Salmonellae* and the Chapman, Stone medium for the *Staphylococci.*

RESULTS

Survival time of the various bacteria incubated at 37°C. in commercial mayonnaise and salad dressing is given (Table 1 and Figs. 1, 2, 3, and 4). Results obtained from the samples incubated at room temperature are not given, since they are the same as those at 37°C.

TABLE 1

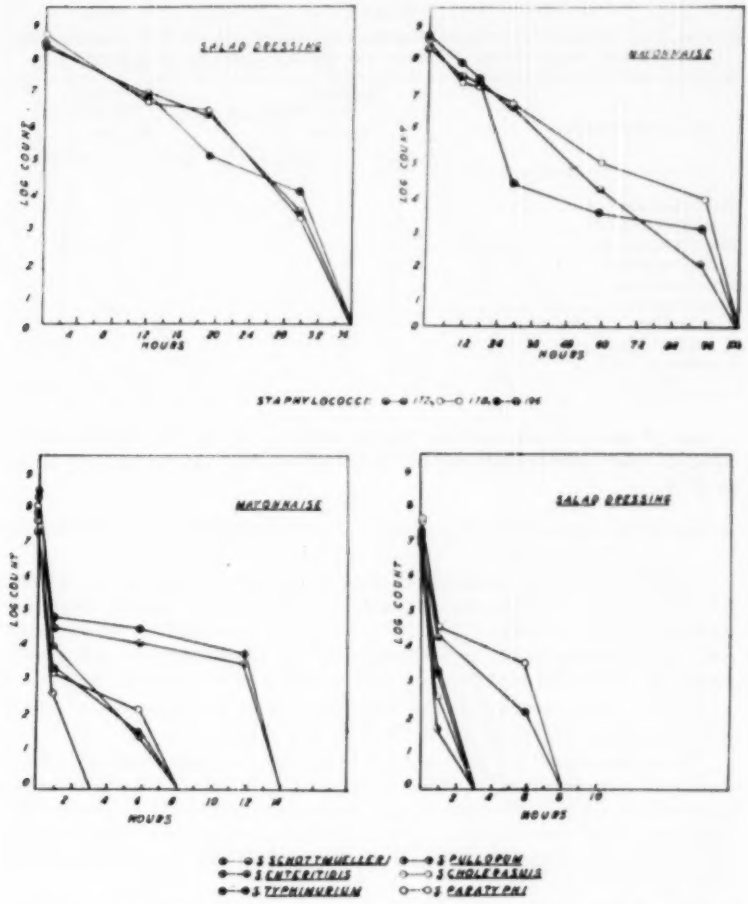
Survival Time of Food-Poisoning Staphylococci and Salmonellae in Commercial Salad Dressing and Mayonnaise

Organism	Mayonnaise (0.48% acid, pH 3.80)	Salad dressing (1.10% acid, pH 3.20)
	hr.	*hr.*
Staphylococcus 172	96	30
Staphylococcus 178	96	30
Staphylococcus 196	96	30
S. schottmuelleri	12	6
S. typhimurium	12	1
S. paratyphi	6	6
S. enteritidis	6	1
S. choleraesuis	1	1
S. pullorum	6	1

II. VIABILITY OF FOOD-POISONING *STAPHYLOCOCCI* AND *SALMONELLAE* IN SALAD DRESSING AND MAYONNAISE, MADE WITH FRESH EGG YOLK AND EMULSOL EGG YOLK IN THE LABORATORY

To determine whether there would be any difference in viability of the various strains of *Staphylococci* and *Salmonellae,* when inoculated into commercial and laboratory-made salad dressing and mayonnaise, batches of each were made in the laboratory and seeded with the various strains of each organism being studied. Yolks of fresh eggs and emulsol egg yolks (prepared commercially with salt and frozen under controlled conditions) were used in these experiments.

One ml. of a 24-hour culture of each of the bacteria was added to 50 gm. of the fresh and emulsol egg yolk, respectively. After 24 hours' incubation at 37°C., the salad dressing and mayonnaise were made, using the inoculated yolks. The laboratory-made salad dressing and mayonnaise



FIGS. 1, 2, 3, and 4. Viability of food-poisoning *Staphylococci* and *Salmonellae* in commercial salad dressing and mayonnaise.

contained the same percentage of oil, salt, and titratable acidity as the average commercial product. Samples were incubated at 37°C. and room temperature, and cultured at various time intervals on the respective media.

RESULTS

Survival time of the various bacteria in mayonnaise and salad dressing prepared with fresh and emulsol egg yolks are given (Table 2 and Figs. 5, 6, 7, 8, 9, 10, and 11).

TABLE 2

Survival Time of Food-Poisoning Staphylococci and Salmonellae in Salad Dressing and Mayonnaise Prepared With Fresh and Emulsol Egg Yolks in the Laboratory

Organism	Mayonnaise (0.51% acid, pH 4.0)		Salad dressing (1.02% acid, pH 3.30)	
	Egg yolk		Egg yolk	
	Fresh	Emulsol	Fresh	Emulsol
	hr.	*hr.*	*hr.*	*hr.*
Staphylococcus 176	78	72	48	16
Staphylococcus 196	78	72	48	16
Staphylococcus 172	20	72	48	16
S. schottmuelleri	18	12	8	1
S. typhimurium	6	12	6	1
S. paratyphi	6	12	8	1
S. enteritidis	18	12	8	1
S. choleraesuis	6	12	3	1
S. pullorum	18	8	8	1

As in the previous section, results obtained from the samples incubated at room temperature are not given, since they are the same as those at 37°C.

III. EFFECT OF ACETIC ACID UPON SURVIVAL OF FOOD-POISONING *STAPHYLOCOCCI* AND *SALMONELLAE* IN SALAD DRESSING AND MAYONNAISE

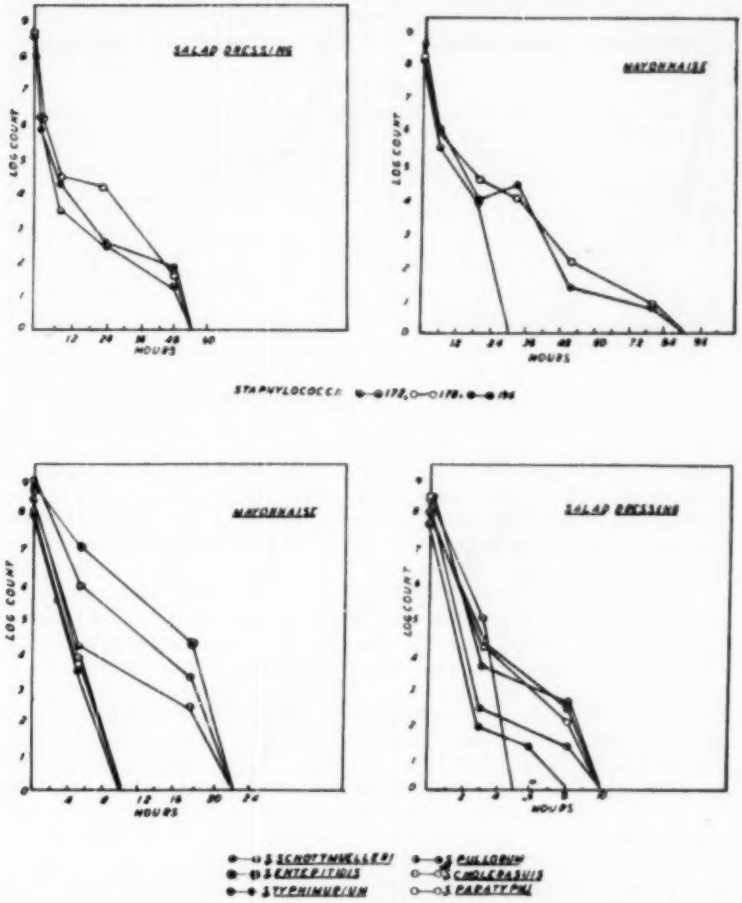
Acetic acid which is added to salad dressing and mayonnaise in the form of vinegar is expressed as titratable acidity. This acidity for salad dressing ranges between 0.8 and 1.2 per cent calculated as acetic acid, with an approximate pH of 3.3; while the pH of mayonnaise is approximately 4.0, with the acid ranging between 0.4 and 0.7 per cent acetic.

Porter (1946) stated that the minimum pH of the *Salmonellae* varies with the strain, the lowest being 4.5 for *S. paratyphi*.

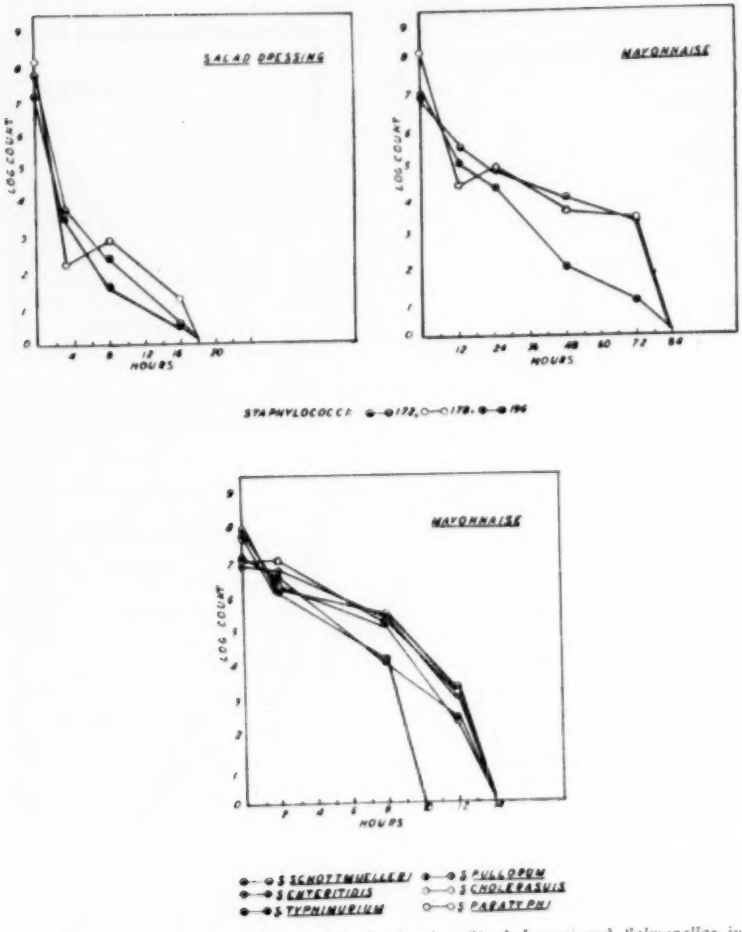
To determine if the per cent acid present in these products affects survival of the bacteria, both salad dressing and mayonnaise were made to contain a lower percentage of acid than commonly found in commercial products. Salad dressing was made to contain 0.4 per cent acid as acetic with a pH of 4.4, and mayonnaise to contain 0.15 per cent acid as acetic with a pH of 5.0.

Before making the products, an attempt was made to adapt the bacteria to an acid medium by culturing them in nutrient broth adjusted to different pH's in the acid range. *S. typhimurium* and *S. schottmuelleri* were found to survive in pH's to and including 4.5 while *S. enteritidis* and *S. paratyphi* survived a pH of 4.7. *S. pullorum*, *S. choleraesuis*, and the three strains of *Staphylococci* would not survive in a medium lower than 5.6.

One ml. of a 24-hour culture of the bacteria grown in nutrient broth having the respective pH's was added to fresh egg yolk and incubated at 37°C. for 24 hours. Salad dressing and mayonnaise were made to contain the amounts of acid previously mentioned and the same percentage of oil and other ingredients as the commercial products.



FIGS. 5, 6, 7, and 8. Viability of food-poisoning *Staphylococci* and *Salmonellae* in salad dressing and mayonnaise made with fresh egg yolk in the laboratory.



FIGS. 9, 10, and 11. Viability of food-poisoning *Staphylococci* and *Salmonellae* in salad dressing and mayonnaise made with emulsol egg yolk.

RESULTS

Survival time of the various bacteria in salad dressing that contained 0.4 per cent acid with pH of 4.4, and in mayonnaise that contained 0.15 per cent acid as acetic with a pH of 5.0 is given (Table 3 and Figs. 12, 13, 14, and 15).

TABLE 3

Survival Time of Food-Poisoning Staphylococci and Salmonellae in Salad Dressing That Contained 0.4 Per Cent Acid, pH 4.4; and Mayonnaise That Contained 0.15 Per Cent Acid as Acetic With a pH of 5.0

Organism	Mayonnaise	Salad dressing
	hr.	hr.
Staphylococcus 178	144	168
Staphylococcus 172	144	120
Staphylococcus 196	144	120
S. schottmuelleri	144	144
S. typhimurium	144	120
S. paratyphi	156	24
S. enteritidis	156	96
S. choleraesuis	156	96
S. pullorum	132	96

DISCUSSION

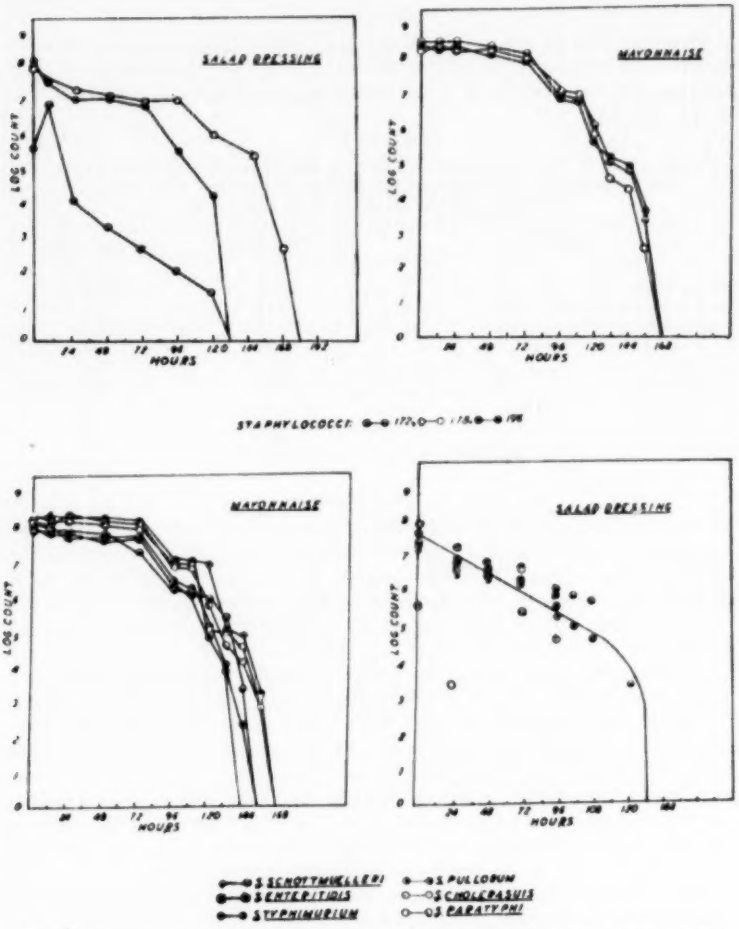
Results obtained when approximately a million bacteria were added per gram of salad dressing and mayonnaise, show that in general the enterotoxigenic strains of food-poisoning *Staphylococci* were more viable in both these products than the *Salmonellae*. Several species of *Salmonellae* were found to be more viable than others. The difference in time ranged from 24 to 144 hours for the different species.

Results also show that both the *Staphylococci* and *Salmonellae* were viable for a greater length of time in mayonnaise than in salad dressing.

When salad dressing and mayonnaise were made with fresh and emulsol-inoculated egg yolks, the total count per gm. of the finished product was approximately a hundred thousand bacteria. Most of the bacteria survived a few hours longer in mayonnaise made with fresh yolk, than in the product made with emulsol yolk. Survival time of all the bacteria in both salad dressing and mayonnaise made with fresh egg yolk was longer than in the same products made with emulsol yolks.

The bacteria in every instance remained viable for a greater length of time in mayonnaise than in salad dressing. A comparison of the amount of the respective ingredients in each product is of interest, since this is apparently the reason for the difference in viability of bacteria in the two products. Previously reported work by Fabian and Wethington (1950) showed the chemical composition of 110 samples of mayonnaise and the different dressings collected from all parts of the United States.

The amount of oil in mayonnaise is considerably greater than in salad dressing so this ingredient can be ruled out as the causative factor. The egg yolks added serve as an excellent source of food for the kind of bacteria used here, as shown by the literature on the subject. The small amount of salt, sugar, and spices added cannot be considered inhibitory in these quantities for the *Staphylococci*, since Nunheimer and Fabian



FIGS. 12, 13, 14, and 15. Viability of food-poisoning *Staphylococci* and *Salmonellae* in salad dressing containing 0.4 per cent acid and mayonnaise containing 0.15 per cent acid.

(1940) have shown that food-poisoning *Staphylococci* were not inhibited by salt until the concentration reached 15 to 17.5 per cent. The germicidal concentration was 20 per cent. For the same strains, they showed it required from 30 to 35 per cent dextrose and from 50 to 60 per cent sucrose to inhibit their growth, while the germicidal concentration of dextrose ranged from 40 to 50 and for sucrose from 60 to 70 per cent, the amount depending upon the different strains.

Certain spices such as mustard, cassia, cinnamon, and cloves do have a germicidal action, as shown by Blum and Fabian (1943), in concentrations of the essential oils ranging in concentration from 1.0 to 0.1 per cent, which is considerably greater than used in salad dressing and mayonnaise. The starch present in salad dressing would not act as an inhibiting agent, since it does not possess any germicidal properties.

All other ingredients in the salad dressing and mayonaise have been eliminated, except the acetic acid added in the form of vinegar. Mayonnaise tested 0.48 and 0.51 per cent acid and had a pH of 3.80 and 4.0, respectively (see Tables 1 and 2), while salad dressing tested 1.10 and 1.02 per cent acid and had a pH of 3.20 and 3.30. Under these conditions survival time for the different strains of the *Staphylococci* ranged from 72 to 96 hours for mayonnaise and from 16 to 48 hours for salad dressing. Survival time for *Salmonellae* ranged from one to six hours in salad dressing and from one to 18 hours in mayonnaise. When acidity was decreased, survival time was increased (shown Table 3) to practically double that shown previously (in Tables 1 and 2). This demonstrated clearly the role played by acetic acid in reducing viability of the different bacteria in these two products.

S. choleraesuis, *S. enteritidis*, and *S. schottmuelleri* not only survived for 96, 96, and 120 hours, respectively, but in salad dressing testing 0.4 per cent acid the bacteria grew as shown by the increased total counts before they started to decline (shown Fig. 15). The three strains of *Staphylococci* also increased before they started to die. In mayonnaise containing 0.15 per cent acid, total counts of all the bacteria remained approximately the same for the first 72 hours, after which there was a decrease.

SUMMARY

Under the conditions of these experiments the following statements appear justified:

The strains of food-poisoning *Staphylococci* tested were more resistant to the conditions found in salad dressing than were the different species of *Salmonellae* tested.

The one ingredient which had the greatest influence on the viability of the bacteria tested was acetic acid. Decreasing the amount of the acetic acid in either salad dressing or mayonnaise increased survival time of the organism studied.

CONCLUSION

Owing to their acid content, mayonnaise and salad dressing are not probable sources of *Staphylococci* or *Salmonellae* food poisoning.

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SPOILAGE IN SALAD AND FRENCH DRESSING DUE TO YEASTS[1]

F. W. FABIAN AND MARY CATHERINE WETHINGTON
Department of Bacteriology and Public Health, Michigan State College, East Lansing, Michigan

[Received for publication, November 14, 1949]

A great deal of spoilage occurred when the manufacturing of salad dressing, mayonnaise, and related products began on a commercial scale. This was owing to a lack of knowledge in making a high quality product which would keep when subjected to unfavorable conditions such as heat, exposure to light, and contact with moisture.

The commonest types of spoilage were separation of oils from the emulsion, rancidity, and off-flavors, fermentation due to yeasts, and spoilage due to molds and bacteria. Today methods of production, selection of materials, and knowledge of emulsions have so improved that spoilage owing to separation of the emulsion, and rancidity, has disappeared almost entirely. Although the conditions for manufacturing the dressing products have improved, spoilage does occur owing to contamination by microorganisms.

During the past two years, 1947-1949, a bacteriological analysis has been made of more than 100 samples of salad dressing, mayonnaise, and related products from all parts of the United States, as previously reported, Fabian and Wethington (1950). Yeasts were not present in large numbers. However, during this same time four manufacturers in different sections of the country found spoilage in their finished products of salad and French dressing. Gassy fermentation in the jars was the only evidence of spoilage. After standing at room temperature for several hours some of the samples foamed over the sides of the jar when the top was removed. The fermentation did not noticeably affect the odor or taste of the products. Furthermore, a chemical analysis showed the amount of acid, salt, oil, and the pH, to be within the range of the unfermented products.

Contamination in one factory was so bad the factory had to close down and remain closed until the machinery, as well as all the other equipment, was disassembled and thoroughly washed and cleaned. Bacterial analysis of the ingredients showed no excessive contamination which would account for fermentation in the final product. The obvious conclusion was that contamination was due to the machinery.

METHOD

Bacterial examination of the samples yielded a pure culture of yeasts. In the case of the salad dressing the yeast was best isolated on Trommer's malt agar that contained 30 per cent dextrose, while the same medium with 15 per cent dextrose was better for isolation of yeasts from the French dressing. Difco's potato dextrose and malt agars were also used, but a longer time was required for growth and the number of colonies isolated per gm. was not as great.

[1]Journal Article No. 1,095 (n.s.).

Yeasts isolated from salad dressing on Trommer's malt extract agar, Fabian and Withington (1950), to which 30 per cent dextrose had been added, readily adapted themselves to a concentration of 15, then five, and finally one per cent dextrose concentration in Trommer's malt extract agar, while yeasts isolated from the French dressing became adapted to five and then one per cent dextrose concentration in the same medium.

Yeasts isolated from all three samples of fermented material were identified as *Zygosaccharomyces*. So far they have not been classified as to species.

RESULTS

The number of yeasts isolated from the two samples of salad dressing and French dressing is given (Table 1).

TABLE 1

Number of Yeasts Per Gram of Spoiled Salad and French Dressing[1]

Product	Sample No.	Trommer's malt agar	Potato dextrose agar	Difco malt agar
French dressing	91	61	21	30
French dressing	90	341	172	45
Salad dressing	89	110	52	35
Salad dressing	88	250	100	85

[1] Yeast plate counts per gm.

DISCUSSION

While this work was in progress Mrak and Williams (1949) reported a yeast, *Zygosaccharomyces globiformis*, as the causative agent of gassy fermentation in salad dressing. They found that contamination was due to improperly cleaned and unsanitized machinery.

This case was analogous to one case of spoilage in salad dressing reported here. All ingredients were free from excessive contamination and the machinery was found to be implicated. When the machinery was properly cleaned and sanitized, the trouble disappeared.

Paprika was strongly suspected in one case of spoiled French dressing, since spoilage occurred when the company used a new batch of paprika for making the dressing. Unfortunately, they had disposed of all this particular batch, so none was available for analysis. Subsequent samples from new batches sent for analysis showed no yeasts present. In the other two cases of spoilage, companies having the spoilage had no idea of the cause.

Yeasts from all four cases of spoiled dressings could still be isolated at the end of six months. Cultures of these yeasts inoculated into unfermented jars of the dressings caused fermentation within a short time. This would indicate that either they had become acclimated to conditions found in the dressings, or else these yeasts do not need to become adjusted to conditions existing in the dressing since the conditions are already to their liking.

Yeasts belonging to the genus *Zygosaccharomyces* have been found to cause spoilage in other food products. Fabian and Quinet (1928) isolated a large number of species from spoiled honey. Lochhead and Heron (1929)

studied the fermentations of stored honey and found spoilage in each case to be due to a yeast of the genus *Zygosaccharomyces*.

SUMMARY

Two samples of French and two of salad dressing, of over 100 analyzed, were found to be spoiled due to a yeast fermentation.

A yeast belonging to the genus *Zygosaccharomyces* was isolated and proved to be the cause of fermentation in all cases.

One case of spoilage was due to improperly cleaned and unsanitized machinery. Another was thought to be due to contaminated paprika, although this was not proved. No reason could be assigned for the source of contamination in the other two cases.

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BACTERIAL AND CHEMICAL ANALYSES OF MAYONNAISE, SALAD DRESSING, AND RELATED PRODUCTS[1]

F. W. FABIAN AND MARY CATHERINE WETHINGTON
Department of Bacteriology and Public Health, Michigan State College, East Lansing, Michigan

[Received for publication, November 14, 1949]

Widespread use of salad dressing, mayonnaise, and related products has made them important food items. As with other prepared foods, there are possibilities of microorganisms gaining entrance during the process of their manufacture. However, these products contain a high percentage of acid added in the form of vinegar, and since comparatively few species of bacteria are able to survive in a very acid medium it seems improbable that many would be present for any great length of time.

At the present time a definition and standard of identity exists for mayonnaise. There are no standards governing production of other products such as tartar sauce, salad, and French dressing, which doubtless is one reason why there is a wide variation in the amount of basic ingredients used in their manufacture. This is shown in the section on the chemical analysis of the samples (Tables 6 to 10, inclusive).

Bacterial and chemical analyses were made on a large number of samples sent through the courtesy of the Mayonnaise and Salad Dressing Manufacturers' Association, Inc. This Association requested their members throughout the country to send samples to this laboratory to determine the viable microorganisms in the finished product. Different media were employed to determine which ones were the most satisfactory as standard media for bacterial analysis of these products.

METHOD

Owing to the consistency of the various products, different methods were tried for making a bacteriological analysis of them. The best suspension was obtained by adding 20 gm. of the product to 10 ml. of sterile, distilled water and agitating the contents thoroughly and vigorously, either by a mechanical shaker or by hand, for from five to 10 minutes—depending on consistency of the product. Another method tried was the addition of solubilizing agents, such as the Tweens, to the dilution water to produce a more homogenous mass in water. Repeated platings on the different media showed no added advantage, so far as could be determined.

MICROBIOLOGICAL ANALYSIS

A number of different media was used in order to culture the various forms of microbial life that might be present. This was desirable since the work was exploratory in nature.

Over 100 samples of salad and French dressing, mayonnaise, and tartar sauce were analyzed (using the media shown Table 1). The different media were used to determine in so far as possible the microbial flora of the several condiments now so commonly used by the American public.

[1]Journal Article No. 1,096 (n.s.).

TABLE 1

Media Used for Determining Different Kinds of Microorganisms in Salad and French Dressing, Tartar Sauce, and Mayonnaise

Media No.	Media name	Type microorganism to determine	Reference No.
1	Dextrose agar (Difco's)	Total plate count	1932
2	Standard milk agar	Total plate count	1948
3	Dextrose tryptone agar (Difco's)	Thermophilic flat sour bacteria	1933
4	Chapman, Stone agar	Staphylococci and total plate count	1948
5	Eosin methylene blue agar (Difco's)	Coliform bacteria	1916 and 1918
6	Starr's spirit blue agar	Lipolytic bacteria	1941
7	Special media No. 1	Total plate count	.....[1]
8	Potato dextrose agar (Difco's)*	Yeasts and molds	1948
9	Malt extract agar (Difco's)	Yeasts and molds	1919
10	Malt (Trommer's) extract agar	Yeasts and molds	.....[2]

[1] Composition of Special Medium No. 1. [Medium No. 7 (Table 1)]. Glucose tryptone yeast extract agar:

Difco's tryptone	10 gm.
Difco's yeast extract	5 gm.
Difco's beef extract	3 gm.
Difco's di-potassium phosphate	1 gm.
Difco's glucose	1 gm.
Difco's agar	15 gm.
Difco's water	1,000 ml.
Difco's brom-cresol purple	0.5 pct.

[2] Malt (Trommer's) Extract Agar [Medium No. 10 (Table 1)]:

Trommer's malt extract	30 gm.
Agar	20 gm.

*Two ml. of a sterile one to 10 dilution of U.S.P. lactic acid (85 per cent) per 100 ml. were added to the liquefied medium immediately before use.

All the samples received were plated on all the media (shown Table 1). Results are found (Tables 2 to 5).

RESULTS

The number of microorganisms isolated from samples of salad and French dressing, mayonnaise, and tartar sauce was small (shown Tables 2 to 5). Only a few of the samples contained counts sufficiently high to be considered of bacteriological importance. The bacteria isolated were identified microscopically as sporogenic rods, gram-positive *Cocci, Diplococci,* and asporogenic *Bacilli.* Counts were low in practically all cases for all products, but salad dressing and tartar sauce contained the lowest number, doubtlessly owing to the high acidity in these products.

The aerobic, sporogenic rods which appeared on the plates were the result of contamination of the ingredients, and machinery and utensil contamination. Results obtained by Fabian and Orloff (1949) showed that these organisms are present in abundance on fresh cucumbers, but that gradually they die and cause no further trouble after the cucumbers are placed in salt and acid and are pasteurized.

TABLE 2

Microbial Analysis of Salad Dressing Using 10 Different Media[1]

Code No.	Medium No., Table 1									
	1	2	3	4	5	6	7	8	9	10
S 1	0	1	0	0	0	0	1	0	2*	3*
S 2	1*	1*	0	2	0	0	2*	2y	2*	2*
S 3	1	1	0	1	0	0	2*	0	1*	2*
S 4	1	2	0	1	0	0	0	0	0	0
S 5	0	0	0	2	0	0	0	0	0	0
S 6	0	1	0	0	0	0	0	0	0	0
S 7	0	0	0	0	0	0	0	0	0	2*
S 8	0	0	1*	2	0	0	1	2*	2*	0
S 9	1	2	0	3	0	0	3(1)	0	0	0
S 10	0	2	0	2	0	0	3	0	0	0
S 11	0	2	0	1	1	0	3	0	0	2y
18 D	1	1	0	0	0	0	0	0	0	0
115312	2	4	1	8	3	0	6	0	0	0
16 C	2	0	0	0	0	0	3	0	0	2*
M 197	0	0	0	0	0	0	0	0	0	0
459	17	42	2	125	6	5	25	0	0	0
J11C3F5	1	3	0	3	1	0	3	0	0	2*
A 9	0	0	0	1	0	0	0	0	0	y
825	3	6	1	5	1	1	6	0	0	0
S 12	0	0	0	0	0	0	0	y	0	y
S 13	0	2	0	4	0	0	3	0	0	0
S 14	0	3	0	4	0	0	2	0	2*	0
S 15	1	2	0	3	0	0	1	0	0	3*
S 16	0	5	0	0	0	0	3	2	0	0
S 17	0	0	0	2	0	0	0	0	0	0
825(a)	6	15	1	4	0	0	10	0	0	0
825(b)	3	10	0	5	0	0	15	0	0	0
H9294	0	0	0	0	0	0	0	0	0	0
48-48-2(a)	0	0	0	0	0	0	3	0	0	0
48-48-2(b)	0	0	0	0	0	0	5	0	0	0
D583F1(a)	0	0	0	2	0	0	1	0	0	0
D583F1(b)	0	0	0	0	0	0	0	0	0	0
No. 2	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	100y	85y	250y
89	0	10	0	0	0	0	0	52y	35y	110y
B 3	89	210	0	72	0	0	75	0	0	0
B 4	0	10	0	0	0	0	4	0	0	0
B 5	5	25	0	10	0	0	20	0	0	0

[1] Bacterial plate count per gm. *=mold. ( )=acid. y=yeast.

TABLE 3

Microbial Analysis of French Dressing Using 10 Different Media[1]

Code No.	Medium No., Table 1									
	1	2	3	4	5	6	7	8	9	10
D 1	0	2	0	3	0	0	2	0	0	0
D 2	0	0	0	0	0	0	0	0	0	0
D 3	2	4	0	6	0	0	7	0	0	0
D 4	0	2	2	4	0	2	3	2*	2*	4*
D 5	0	2	0	3	0	0	6	0	0	0
D 6	0	0	0	0	0	0	0	0	0	0
D 7	0	8	0	10	0	0	15(3)	0	0	0
D 8	4	10	2	8	0	0	10	0	0	0
D 9	0	5	2	2	0	0	6	0	0	0
D 10	1	4	0	0	0	0	5	0	0	0
D 11	3	8	0	6	0	0	4	0	0	0
D 12	2	4	0	1	0	0	2	0	0	0
D 13	0	5	0	4	0	0	6	0	0	0
D 14	2	2	0	0	0	0	0	0	0	0
D 15	6	8	1	10	0	0	12	0	0	3*
20-E	0	0	0	2	0	0	0	0	0	0
22-F	0	0	0	0	0	0	0	0	0	0
D4Z26M7	18	41	2	75	10	6	41	0	0	0
D 16	2	0	0	2	0	0	3	0	0	0
D 17	2	3	0	4	0	0	4	0	0	0
D 18	0	15	0	2	0	0	10	0	0	0
B 10	0	0	0	0	0	0	0	0	0	0
91	0	10	0	0	0	0	0	21y	30y	81y
90	0	10	0	0	0	0	0	172y	45y	341y
20-E	0	4	0	2	0	0	0	0	0	0
H9294(a)	0	2	0	0	0	0	0	0	0	0
H9294(b)	0	0	0	0	0	0	0	0	0	0
P3R4T2(a)	450	250	80	480	200	50	250	0	0	0
P3R4T2(b)	120	410	50	350	20	60	100	0	0	0

[1] Bacterial plate count per gm. *=mold, ( )=acid, y=yeast.

TABLE 4

Microbial Analysis of Mayonnaise Using 10 Different Media[1]

Code No.	Medium No., Table 1									
	1	2	3	4	5	6	7	8	9	10
M 1	1	4	0	4	0	1	2	0	0	1*
M 2	1	3	0	3	0	1	31	0	0	0
M 3	1	10	0	8	2	2	6	0	0	0
M 4	0	4	0	2	0	0	0	0	0	1*
M 5	0	1	0	0	0	0	0	0	0	0
M 6	0	0	0	0	0	0	0	0	0	0
M 7	0	1	0	0	0	0	2	0	0	0
M 8	0	1	0	2	0	0	0	1*	0	3*
M 9	0	2	0	0	0	0	0	0	1*	3*
M 10	0	8	1	5(2)	0	0	6(3)	0	0	0
M 11	3	2	2	7	0	2	4	y	y	y
M 12	1	3	0	3	0	0	0	0	0	0
M 13	1	3	1	3	0	0	6	0	0	0
14-B	2	1	0	2	0	0	0	0	0	0
M 197	16	20	3	22	0	8	32	0	0	y
E 10	5	2*	0	10	0	0	3	2*	0	4*
365	9	13	1	17	1	4	4	0	0	0
12-A	0	2	0	2	0	2*	2	2*	3*	5*
E 10	2	0	1	13	0	2	10	0	0	0
X24A1B2	2	2	0	2	0	0	2	0	0	0
A 2	12	10	0	11	0	0	20	0	0	0
A 1	0	20	0	14	0	0	0	0	0	0
08H7C3	0	0	0	0	0	0	0	0	0	0

[1] Bacterial plate count per gm. *=mold, ( )=acid, y=yeast.

TABLE 5

Microbial Analysis of Tartar Sauce Using 10 Different Media[1]

Code No.	Medium No., Table 1									
	1	2	3	4	5	6	7	8	9	10
F 2	2	9	0	9	0	0	15	0	0	0
F 3	2*	2*	1*	8	0	2*	10	2*	3*	4*
F 4	0	0	0	0	0	0	1	0	0	0
F 5	0	1	0	1	0	0	1	0	0	0
F 6	0	4	0	4	0	4	6	0	0	0
F 7	0	1	0	3	0	0	2	0	0	0
24-G	1	3	0	2	0	0	2	0	0	0
26-H	1	0	0	5	0	0	3	0	0	0
C 10	6	0	2	35	0	3	36	0	0	0
C 9	2	0	0	1	0	0	5(3)	0	0	y
48-48-3(a)	0	0	0	0	0	0	0	0	0	0
48-48-3(b)	0	0	0	0	0	0	0	0	0	0

[1] Bacterial plate count per gm. *=mold, ( )=acid, y=yeast.

No thermophilic bacteria were found, although considerable starch is used in making salad dressing and most starch is known to contain thermophiles. However, these results are not surprising, since Erickson and Fabian (1942) showed that thermophiles were very sensitive to acids. As little as 0.12 per cent acetic acid affected them adversely.

Bacteria were isolated from several samples on the Starr's spirit blue and eosin-methylene blue agars, but upon further identification they were not found to be typical lipolytic or coliform organisms. Since no typical coliform and lipolytic bacteria were found, it is evident these bacteria do not find these products to their liking, or else they were not present originally. Preliminary experiments, performed by inoculating coliform organisms into mayonnaise and salad dressing, showed that eosin-methylene blue agar would be satisfactory for their determination.

DISCUSSION OF MEDIA

The tryptone glucose yeast extract agar (Medium No. 7, Tables 2 to 5) and tryptone glucose beef extract to which 10 per cent sterile skim milk was added (Medium No. 2, Tables 2 to 5) gave similar results on all products. The latter medium is the standard agar recommended in the 9th edition of Standard Methods for the Examination of Dairy Products (1948), and since there was little or no difference between the two media, it is recommended that this standard agar be used for obtaining total plate count on these products. This standard medium is now being used by all laboratories doing milk control work, so it is readily available.

The Chapman, Stone medium (Medium No. 4, Tables 2 to 5), designed primarily for isolation of food-poisoning *Staphylococci*, had two advantages in this work. First, it would pick up any *Staphylococci* if they were present, and second, when a sample contained a large number of aerobic, sporogenic bacteria, they not only grew readily, but distinctly, on this medium. The Chapman, Stone medium contained sufficient salt to inhibit or keep the spreader colonies within due bounds and permitted growth of the other aerobic, sporogenic bacteria. On the other media the spreading

bacteria would cover the entire plate, and not only inhibited many bacteria that would have grown but made it difficult to count those that did grow.

There were few yeasts present in the general run of samples. When they are present and grow, they cause trouble, due to gas production and pressure inside the jars. Three media were used to determine yeasts, viz., Difco's malt extract agar, Difco's acidified potato dextrose agar, and a medium prepared from Trommer's malt extract agar (Media No. 9, 8, and 10, Table 1). The acidified potato dextrose agar and Trommer's malt extract gave practically the same results. There was little to choose between them. The Difco malt extract agar did not give as many colonies as did the other two. In view of this the acidified potato dextrose agar is recommended, since it is available and is recommended in Standard Methods for the Examination of Dairy Products (1948) for determination of yeasts and molds.

There was only one sample (F3, Table 5) that contained sufficient number of molds to be considered as contaminated with molds.

SUMMARY

An analysis of 103 samples of mayonnaise, salad and French dressing, and tartar sauce submitted by the membership of the Mayonnaise and Salad Dressing Manufacturers' Association, Inc., showed the following:

None of the samples contained thermophiles.
There were no coliform organisms present in the samples.
There were no lipolytic bacteria present.
There were few yeasts present in most samples.

Some of the yeasts present belong to the genus *Zygosaccharomyces* and are capable of causing trouble, under proper conditions. The others found were not identified.

There were not many molds present in the samples. Only one sample contained a sufficient number to be considered contaminated.

Standard agar (tryptone-glucose-beef-extract agar), with 10 per cent skim milk added, proved satisfactory for making routine total plate counts.

Acidified potato dextrose agar was as good a medium as any tried, for making routine yeast and mold counts. Special media are necessary to isolate yeasts from fermenting products.

Although no coliforms were found, experiments showed that eosin-methylene blue agar would be satisfactory for determining the coliform count. No lipolytic organisms were isolated, but experiments showed that Starr's spirit blue agar was satisfactory, if an analysis were to be made.

CHEMICAL ANALYSIS

The chemical analysis of the samples submitted included a determination of the pH, titratable acidity, the per cent of salt, moisture, and fat.

METHODS USED

For pH the samples were diluted five times with distilled water and pH determined by a Beckman pH meter using a glass electrode.

Titratable acidity was determined by weighing 10-gm. samples, diluting them to 60 ml. with distilled water, and titrating them with 0.1666 N NaOH. Results were expressed in terms of acetic acid.

Per cent salt was determined according to the method given by Joffee (1942) for determining the amount of salt in salad dressing and mayonnaise. Three gm. of the weighed material were mixed with 30 ml. of distilled water. The chlorides were precipitated with an excess (10 ml.) of 0.1 N silver nitrate in the presence of 10 ml. of nitric acid. The chlorides were titrated with 0.1 N potassium thiocyanate, using a solution of approximately 25 per cent ferric ammonium sulphate as an indicator. The calculation was as follows:

$$\text{Per cent salt} = \frac{(\text{ml. of } AgNO_3 - \text{ml. of KSCN}) \times 0.005845 \times 100}{\text{Weight of sample}}$$

The amount of moisture was determined by drying a three-gm. sample in a vacuum oven at from 65 to 75°C.(149 to 167°F.) until it reached constant weight.

The Bailey, Walker apparatus was used to determine the amount of fatty material present. A three-gm. sample was dried in a vacuum oven at 65 to 75°C. until it reached constant weight. The sample was then extracted with ether for 16 hours, dried, re-weighed, and the per cent fat calculated.

RESULTS

Results of the chemical analysis are given (Tables 6 to 10).

TABLE 6

Per Cent Fat Determined by Use of Bailey-Walker Apparatus

Product	No. samples	Fat (min.)	Fat (max.)	Fat (av.)
		pct.	*pct.*	*pct.*
Mayonnaise	25	71.18	83.36	79.05
Salad dressing	40	21.87	49.87	38.52
French dressing	30	5.25	73.23	35.97
Tartar sauce	15	22.09	51.57	34.20

TABLE 7

Per Cent Moisture

Product	No. samples	Moisture (min.)	Moisture (max.)	Moisture (av.)
		pct.	*pct.*	*pct.*
Mayonnaise	25	10.13	17.27	14.07
Salad dressing	40	30.14	52.00	40.93
French dressing	30	14.75	70.85	39.95
Tartar sauce	15	38.01	60.50	43.76

TABLE 8

Per Cent Salt as Determined by a Precipitation and Titration Method, With Use of $AgNO_3$ and KSCN

Product	No. samples	Salt (min.)	Salt (max.)	Salt (av.)
		pct.	*pct.*	*pct.*
Mayonnaise	25	1.16	1.43	1.39
Salad dressing	40	1.38	1.42	1.40
French dressing	30	1.30	1.58	1.41
Tartar sauce	15	1.35	1.43	1.42

TABLE 9

Titratable Acidity Expressed in Terms of Per Cent Acetic Acid

Product	No. samples	Acetic acid (min.)	Acetic acid (max.)	Acetic acid (av.)
		pct.	*pct.*	*pct.*
Mayonnaise	25	0.30	0.78	0.45
Salad dressing	40	0.82	1.41	0.95
French dressing	30	0.75	2.52	1.20
Tartar sauce	15	0.98	1.65	1.10

TABLE 10

Hydrogen Ion Concentration (pH)

Product	No. samples	Hydrogen ion conc. (min.)	Hydrogen ion conc. (max.)
		pH	*pH*
Mayonnaise	25	3.0	4.1
Salad dressing	40	3.0	3.9
French dressing	30	3.0	4.4
Tartar sauce	15	2.9	3.6

SUMMARY

A chemical analysis of 110 samples of the various commercial samples of salad dressing, mayonnaise, and related products collected from all over the United States showed that:

The greatest variations in chemical composition occurred in the oil and water content of the products.

Mayonnaise had the least, and French dressing the greatest, variation in chemical composition of any of the products analyzed.

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HEAT CAPACITY OF STABILIZED PEANUT BUTTER[1]

T. L. WARD, W. S. SINGLETON, AND A. F. FREEMAN
Southern Regional Research Laboratory,[2]
New Orleans 19, Louisiana

[Received for publication, November 18, 1949]

There is at present a notable lack of reported physical data on peanut butter, despite the obvious utility of such information in the design of peanut butter processing equipment, and the significance of these properties in the production and utilization of the product itself.

In an effort to establish quantitatively the physical characteristics of peanut butter, as part of a program to improve the quality of this peanut product, the heat capacity of a "stabilized" peanut butter was determined by a precise calorimetric method, together with the change in heat capacity, when this product was cooled at different rates. The present report is devoted to a description of the calorimeter and results obtained in two determinations of the heat capacity of average peanut butter cooled at different rates.

SAMPLE

A "stabilized" peanut butter, as recognized by the industry, generally is one in which a hydrogenated vegetable oil, usually peanut oil hydrogenated to an iodine value of about 10, has been dispersed more or less uniformly and in sufficient proportion, so that oil in the peanut butter will not separate under ordinary conditions of shelf-life. Such a product was prepared from No. 1 Grade Spanish peanuts in a 250-pound capacity, batch-type peanut butter pilot plant. The peanuts were roasted for 23 minutes at from 96 to 138°C.(205 to 280°F.), blanched with a split-nut blancher, sorted on a picking table for elimination of the rotten ones and residual material retaining skin, and ground in a peanut butter mill. The ground nuts issued from the mill at approximately 63°C.(145°F.). Percentages by weight of the constituents passed through the grinder were as follows: Roasted, blanched peanuts—97.8 per cent; salt—1.5 per cent; and refined, bleached, and hydrogenated peanut oil [melting point approximately 64.4°C.(148°F.)], and iodine value—5.4 to 0.7 per cent. Analytical data on samples taken at various stages in the processing of this butter are given (Table 1).

APPARATUS

The calorimetric technique and basic principles of the apparatus used have been described in a previous publication, Bailey, Todd, Singleton, and Oliver (1944). The calorimeter was modified, to adapt it to use with a material of high plasticity such as is possessed by peanut butter. The perforated discs of the original calorimeter were replaced by perforated longitudinal vanes (as shown, Fig. 1.). The stationary vanes were soldered to the axially-placed thermocouple well, while the other vanes were

[1] Report of study made under the Research and Marketing Act of 1946.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE 1

Analysis of Peanuts and Peanut Butter

	Moisture content[1]	Oil content		Free fatty acids in extracted oil
		As received	Moisture-free	
	pct.	*pct.*	*pct.*	*pct.*
Raw shelled peanuts[2]	6.0	44.8	47.7	1.1
Roasted peanuts[2]	2.0	47.0	48.0	0.21
Blanched peanuts from picking table[2]	1.8	48.4	49.3	0.24
Peanut butter[3]	1.8	47.3	48.1	0.12

[1] Forced draft oven at 130°C. for three hours.
[2] Prepared in a Henry slicer.
[3] Ground peanuts plus salt and hydrogenated peanut oil.

designed to be removable, to facilitate filling and emptying the calorimeter. The vanes were in good contact with the calorimeter wall and insured rapid conduction of heat to and from the sample. The top disc of the calorimeter was removed during filling, after which it was replaced and sealed with soft solder. The calorimeter and technique of thermal measurement used is accurate to about one per cent, and is capable of detecting the melting of as little as 0.01 to 0.02 per cent of solid fat, or the presence of an equal percentage of crystals in the nearly melted fat.

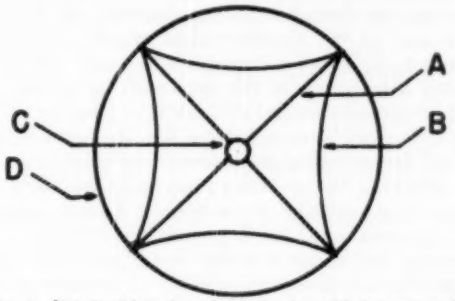

FIG. 1. Detail of interior of calorimeter with top removed.
A—Stationary vanes. B—Removable vanes. C—Thermocouple tube. D—Calorimeter wall.

RESULTS AND DISCUSSION

The specific heat was determined for a sample of stabilized peanut butter heated in the calorimeter to about 80°C.(176°F.) and slowly cooled (about 0.1°C. per minute); and for the sample heated to 80°C. and cooled at an average of 4°C.(39°F.) per minute, from 80°C. to approximately −10°C.(14°F.). Specific heat determinations under both types of cooling were made over the temperature range of 0°C.(32°F.) to approximately 80°C. Results are shown (Table 2).

From the experimental data, an equation was developed for expressing the specific heat, Cp (calories per gm.), of peanut butter, in terms of temperature t, (degrees C.) for the range 20 to 80°C.(68 to 176°F.):

$$Cp = 0.361 + 0.0012\,t$$

TABLE 2

Specific Heats During Slow and Rapid Cooling of a Stabilized Peanut Butter[1]

Slowly cooled sample		Rapidly cooled sample	
Temp.	*Cp*	Temp.	*Cp*
°C.		°C.	
2.27	0.366	2.89	0.397
5.19	0.389	6.18	0.422
9.80	0.416	9.09	0.443
12.65	0.423	11.97	0.498
15.25	0.410	15.41	0.402
18.74	0.385	19.03	0.386
25.33	0.388	30.06	0.403
29.51	0.393	33.24	0.400
32.74	0.401	46.17	0.409
39.96	0.407	49.40	0.419
47.12	0.428	55.52	0.421
54.11	0.433	61.92	0.433
61.57	0.439	67.80	0.441
67.48	0.441	73.88	0.448
74.57	0.453	.....	.....
78.35	0.456	.....	.....

[1] Specific heat, *Cp*, in terms of calories per gm. of substance.

The calculated specific heat of peanut butter differed by not more than ± 0.001 calorie per gm. from plotted experimental values.

The specific heat of the rapidly cooled sample of stabilized peanut butter was found to be about 0.075 calorie per gm. higher than that of the slowly cooled sample, up to the final melting of the hardened fat, which occurred at approximately 12°C.(54°F.). The specific heat of both slowly and rapidly cooled peanut butters was the same at approximately 19°C.(66°F.) and remained equal in further measurements at increasing temperatures, indicating the absence of any solid fat above this temperature. The higher heat capacity of the rapidly cooled sample in the temperature range in which solid fat is present, would seem to indicate that this type of cooling had resulted in the crystallization of part of the fat in a polymorphic form, which was partially or completely different from the crystalline form obtained by slow cooling. It has been shown by Charbonnet and Singleton (1947), among others, that triglycerides in unstable crystalline forms have higher heat capacities than do stable crystalline forms of the same triglycerides. However, the specific heats of both rapidly and slowly cooled peanut butters became equal and increased by the same increments from a temperature of about 19 up to 80°C., a temperature range much broader than that normally used in the industrial production of peanut butter. Rate of cooling of peanut butter during normal processing would therefore have no effect on its thermal properties, unless it was cooled to temperatures below 19°C.

SUMMARY

A calorimeter suitable for investigation of the heat capacity of peanut butter has been described.

The specific heat of a stabilized peanut butter has been determined for both a slowly cooled and a rapidly cooled sample, over a range of 0 to

80°C., and an equation derived for expressing the specific heat from 20 to 80°C., which is: $Cp = 0.361 + 0.0012\ t$.

Rapid cooling of peanut butter appeared to cause a polymorphic change in the hard fat content of peanut butter, yielding an unstable form with a heat capacity higher than that of slowly cooled peanut butter.

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RUTIN CONTENT OF ASPARAGUS[1]

A. E. STEVENSON
Research Department, Continental Can Company, Inc., Chicago, Illinois

[Received for publication, December 20, 1949]

The presence of rutin in asparagus has been previously reported by DeEds and Couch (1948). Also, Balansard and Raybaud (1937) reported the presence of heterosides in asparagus, and Campbell (1939) reported the presence of a flavonol glucoside. Previous to the publication of the article by DeEds and Couch, this laboratory, during an investigation of the changes of color in canned asparagus, had independently identified the glucoside present as rutin. Our work on edible asparagus showed the tip contained a larger percentage of rutin than did the remainder of the edible stalk. Some figures on the amount found in the edible stalk are given (Table 1). These samples represent asparagus grown for the

TABLE 1

Rutin in Edible Asparagus

Sample No.	Rutin	Solids
	pct.	*pct.*
1	.041	7.53
2	.067	8.43
3	.039	8.54
4	.040	7.86
5	.037	6.62
6	.098	7.06
7	.026	8.26

market in 1949, in the vicinity of Chicago, Illinois. Distribution of the rutin between the tips and remainder of the edible stalk was determined in the case of the first three samples and is shown (Table 2).

The method used for the determination of the rutin content was as follows: Two hundred gm. of the sample were extracted in a Waring Blendor with somewhat more than an equal weight of acetone, followed by filtering on a Buchner funnel. The residue was returned to the Waring

[1] Presented before the Division of Agricultural and Food Chemistry at the 116th Meeting of the American Chemical Society, Atlantic City, New Jersey, September, 1949.

TABLE 2

Distribution of Rutin in Edible Asparagus

Sample No.	Tips		Remainder edible stalk	
	Rutin content	Total stalk	Rutin content	Total stalk
	pct.	*pct.*	*pct.*	*pct.*
1	0.093	29.3	0.020	70.7
2	0.138	31.3	0.035	68.7
3	0.078	33.8	0.019	66.2

Blendor and extracted twice more, using each time 200 to 250 ml. of 70 per cent acetone. The combined acetone solutions were extracted twice with ether, to remove ether-soluble materials, including chlorophyll and carotene. Ether and acetone were removed from the solution by evaporation to from 200 to 250 ml., and the rutin precipitated from the aqueous solution by means of a 10 per cent aqueous solution of lead subacetate. The precipitate was filtered off, washed with a small amount of distilled water, transferred to a beaker, disintegrated in methyl alcohol, and the lead salts decomposed by adding a two per cent solution of sulphuric acid in methyl alcohol. The sulphuric acid solution was added gradually with stirring until the pH was 3. The presence of a small amount of anthocyanin in the product, which acts as an indicator, assists in adjusting the pH. The precipitate was filtered off, washed with methyl alcohol, the combined alcohol solutions neutralized with $BaCO_3$, and evaporated nearly to dryness in the presence of an excess of $BaCO_3$. The residue was taken up with methyl alcohol and the insoluble material filtered off. The filtrate was evaporated to a few ml., the rutin allowed to crystallize by standing several hours in a cool place, filtered off, washed with a small amount of distilled water, allowed to air-dry, and weighed.

This method should be considered as giving the approximate rutin content rather than being a quantitative method. The rutin crystals are contaminated with small amounts of an anthocyanin which has been identified by Robinson and Robinson (1934) as a cyanidin diglycoside. On the other hand, it is not certain that all of the rutin is precipitated by the lead subacetate solution. Results of duplicate determinations frequently varied as much as 10 per cent. It is probable that the figures given are on the low side.

In determining the rutin in the plant after the edible stage was past, the procedure was the same as above, up to the point of precipitating with lead subacetate. Instead, the solution after evaporation of ether and acetone was further evaporated at a temperature of 80 to 90°C.(176 to 194°F.) to a small volume, when the rutin crystallized out in a quite pure condition. The crystals were filtered off, washed with a small amount of water previously saturated with rutin, and allowed to air-dry.

As the asparagus plant passes from the edible stage to the more mature stage there is considerable increase in the rutin content, particularly after the cladophylls have developed. Figures on the rutin content of the asparagus plant collected in the vicinity of Chicago, after it had passed the edible stage and at different periods of the year, are given (Table 3).

All samples were collected in the vicinity of Chicago, Illinois. Samples 1 to 11, inclusive, were from the same field from which, both in 1948 and in 1949, edible asparagus previously had been harvested. Samples 7a and 7b were collected a few days after a heavy frost, 7b being brownish-yellow in color, while 7a still showed a trace of green. A portion of the cladophylls had shattered from both of these samples. Sample No. 8 was collected approximately two weeks after harvesting, for the fresh market had ceased. Sample No. 12 was from another field from which asparagus previously had been harvested for the fresh market. Samples 13, 14, and

TABLE 3

Rutin Content of Asparagus

Sample No.	Date collected	Discarded	Rutin in portion analyzed	Solids in portion analyzed	Rutin, moisture-free basis
		pct.	*pct.*	*pct.*	*pct.*
1	7/19/48	.....	.71	.....	.....
2	7/24/48	.....	.66	.....	.....
3	8/26/48	25.0	.71	.....	.....
4	9/ 4/48	19.7	.77	37.15	2.07
5	9/25/48	32.3	.64	35.35	1.81
6	10/ 1/48	34.8	.61	40.37	1.51
7a	10/22/48	31.3	.54	66.88	0.81
7b	10/22/48	27.0	.94	71.49	1.31
8	7/ 4/49	30.8	.52	25.45	2.04
9	7/19/49	24.8	.56	30.55	1.83
10	8/ 3/49	29.0	.56	30.92	1.81
11	8/29/49	34.6	.63	33.99	1.85
12	9/14/49	32.0	.90	36.44	2.47
13	6/22/49	35.8	.92	35.27	2.61
14	6/25/49	35.0	.71	33.56	2.12
15	6/29/49	29.1	1.01	41.79	2.42

15 were from small plots from which fresh asparagus had not been harvested and the plants were, therefore, well developed at the time they were collected.

It will be noted that of the samples collected from the same field in 1948 the maximum rutin content occurred early in September, or approximately two and one-half months after harvesting for the fresh market had ceased, although there is not much variation during the period over which samples were collected. Samples (8, 9, 10, and 11) collected in 1949 from this same field, do not show as high a percentage of rutin as in 1948. Samples (13, 14, and 15) from small plots from which asparagus had not been harvested for the fresh market showed the highest rutin content. This may be due to the fact that the plants were scattered, permitting more vigorous growth.

In preparing samples for analyses, the large center stems were discarded; also the larger blanching stems. The remaining material was chopped into small pieces in order to secure a uniform sample. The discarded material contained practically no rutin. A sample collected September 4, 1948, was separated into three portions—viz., large central stems, 19.6 per cent; small stems, 10 per cent (no cladophylls attached); and "very small stems," with the cladophylls attached, 70.4 per cent. (The "very small stems" were severed from the branch just below the point where the first cladophylls were attached.) The large stems were discarded; the small stems contained 0.28 per cent rutin, and the very small stems with cladophylls attached contained 0.89 per cent, both on the "as collected" basis.

Similar data were obtained on a sample collected June 29, 1949. The discarded portion amounted to 35.4 per cent, in which rutin was not determined; the small stems, to 18.8 per cent, in which the rutin content was 0.22 per cent; and the very small stems with cladophylls attached.

to 45.8 per cent, in which the rutin content was 1.20 per cent, both on the "as collected" basis.

In order to establish definitely that the product from the mature asparagus plant was rutin, the usual identification tests were made. Quercetin, rhamnose, and glucose were formed on hydrolysis. Using the spectrophotometric method developed by Porter, Brice, Copley, and Couch (1947), $k_{362.5}$ was found to be 30.68 for crude rutin, separated by the method previously described from asparagus collected in August, 1948. Using the formula $R = 100\ k_{362.5}/33.22$, the percentage of rutin in the crude product was found to be 92.4.

Couch, Naghski, and Krewson (1946) have shown that the drying of buckwheat causes considerable decrease in rutin content. An experiment was carried out to determine the effect of drying on rutin content of asparagus. After removal of the large central stems and chopping to insure a uniform sample, a quantity was divided into four lots. One lot was analyzed without drying, one lot dried four and one-half hours at 70°C.(158°F.), one dried two and one-half hours at 100°C.(212°F.), and one lot dried one hour at 110°C.(230°F), the drying in all instances being carried out in a forced-circulation, hot-air oven. Moisture contents of the last three dried samples, as determined by drying in vacuum at 70°C. were 5.18, 2.46, and 2.30 per cent, respectively. Rutin contents on a moisture-free basis are as follows:

Original, undried	2.74 per cent
Dried four and one-half hours at 70°C.	2.31 per cent
Dried two and one-half hours at 100° C.	2.14 per cent
Dried one hour at 110°C.	2.09 per cent

The per cent losses are 12, 21.9, and 31, respectively, which are materially lower than those reported by Couch *et al.* (1946) when drying buckwheat at similar temperatures. A lot from another source dried for 44 hours at 50 to 55°C.(122 to 131°F.) in a forced-circulation, hot-air oven showed a rutin content of 1.77 per cent on a moisture-free basis, as compared with 1.82 per cent in the original.

The presence of rutin in edible asparagus is of interest as being the only recorded instance of the presence of this compound in a part of a plant used as food. The amount of rutin in mature asparagus is sufficient to make the plant a possible commercial source of this compound. There is some doubt, however, whether asparagus could be harvested for the fresh market and the mature plant cut at a later date for rutin production, without greatly reducing production of edible asparagus the following year. The plant during its growth, after the cessation of harvesting edible asparagus, stores in the rootstock food used the next year in production of edible stalks. Perhaps harvesting late in the fall would not be damaging to the following year's crop of edible asparagus.

Summarizing, by precipitation with lead subacetate and recovery of rutin from the lead salt, the rutin content of edible asparagus, based on a limited number of analyses, was found to vary from approximately 0.025 to 0.10 per cent, with the percentage in the tip being three to four times as high as in the remainder of the edible stalk. Rutin content of the

mature asparagus plant collected in the vicinity of Chicago was found to vary from 0.52 to 1.01 per cent on the portion of the plant analyzed, which constituted approximately 70 per cent of the entire plant. The rutin content of the mature asparagus plant is sufficiently high to make it a possible commercial source of this compound.

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EFFECT OF BAKING PAN MATERIAL ON HEAT PENETRATION DURING BAKING AND ON QUALITY OF CAKES MADE WITH FAT

HELEN CHARLEY

Department of Foods and Nutrition, Oregon State College, Corvallis, Oregon

[Received for publication, December 20, 1949]

It has been shown that the material from which an oven utensil is made influences baking results. In other studies which deal with oven utensils made from different materials, attention seems to have been focused on the thermal efficiency of the pans. There has been little or no consideration of the effects of the different pans upon the resultant baked product.

Of the materials and finishes used by Gartrell (1930) in her study of the thermal efficiency of pans in a household type electric oven, the pan with two coats of white enamel on the inside and one coat of dark on the outside proved most efficient. Considering the efficiency of this pan an arbitrary 100 per cent, the others rated in descending order as follows: two coats of white enamel inside and out 87.7; glass 81.9; steel, 67.8; aluminum, 58.1; copper, 57.6; and tin, 46.8. Cornehl and Swartz (1931) determined the ratio of the heating effected in the utensil to the heat supplied to the oven and reported efficiencies of 24.7 per cent for cast iron, 22.4 for china, 21.9 for glass, 19.2 for enameled iron, 17.7 for blackened aluminum, and 13.6 for aluminum. Using the rate of evaporation of water as a criterion, Phillips and Nordberg (1934) reported that in an electric oven at a temperature of 204°C.(400°F.) the heating rate for a shiny tinware utensil was 47.4 per cent of that for a similar utensil made of glass. Reed, Floyd, and Pittman (1937) used angel food cake batter as their test material, and the maximum internal temperature reached in the cake as a measure of the thermal efficiency of the pans they studied. They reported the highest thermal efficiency for cake pans made from glass, followed by those made from enameled iron, Russian iron, aluminum, and tin. They found that cakes baked in pans made from the first three materials had crusts that were too brown and too thick, while those from aluminum and tin had light-brown crusts. They further observed that the glass pan produced a tender cake, as did the aluminum and the tin, while the enameled iron and the Russian iron pans yielded cakes with tough crumb.

Of the five works reviewed above, only the last one deals with the effects of the baking pan material upon quality of the baked product. No work has been reported dealing with effects of the material used for the baking pan upon quality of cakes made with fat.

Cakes made with fat increase in volume and improve in quality as the baking temperature increases from 155 to 185°C.(311 to 365°F.), as shown by Stone (1934). Presumably more rapid heat penetration, with increasing oven temperature, causes cake batter to set before internal agitation harms the structure of the cake. All studies previously cited indicate that

the character of the baking pan influences its thermal efficiency. Thus the baking pan, as well as the oven temperature, could influence rate of heat penetration in batter baked therein, and presumably would affect the quality of the baked product. The study here reported was undertaken to test this theory.

Plain cake batter was baked in pans made from eight different metals, and from glass. Data were obtained on rate of heat penetration in the batter during baking, on length of baking period, and on quality of the baked cakes.

EXPERIMENTAL PROCEDURE

Baking Pans: Small loaf pans 5 x 3 x 2¾", with straight sides and a capacity of 680 ml., were made from metals available at the time the study was undertaken. Metals from which the eight pans in this series (Series I) were made included aluminum with a shiny finish, anodized or electrolytically oxidized aluminum with a dull finish, tinned iron, iron with a lacquered or japanned finish, rough sheet iron, hot rolled steel with a black iron oxide coating on the surface, copper, and stainless steel.

It seemed desirable to compare a glass baking pan with the metal ones. A glass pan of the same dimensions as the small metal pans in Series I was not available and it was impossible to have one made. Therefore, a small rectangular oven-glass baking pan 9⅞ x 6 x 1⅜", with a capacity of 1,550 ml., was selected, and a second pan of the same size and shape made from the hot rolled steel which had been used to make one of the smaller metal pans. These two larger pans constituted Series II. Data on weight, thickness, and nature of the surface of each pan are found (Table 1).

TABLE 1

Some Characteristics of Cake Pans Used

Material	Nature of surface	Thickness	Weight
		mm.	*gm.*
Series I			
Aluminum	Bright, smooth	0.65	79.5
Anodized aluminum	Dull, rough	0.74	89.1
Tinned iron	Bright, smooth	0.25	90.1
Japanned iron	Dark, smooth	0.24	92.2
Steel	Dark, dull	0.35	129.1
Sheet iron	Rough	0.73	243.7
Copper	Bright, smooth	0.48	199.7
Stainless steel	Bright, smooth	1.57	490.5
Series II			
Glass	Transparent	5.17[1]	879.1
Steel	Dark,dull	0.35	227.5

[1]Average thickness.

Formula Used: For testing the smaller metal pans (Series I), batter made from the following plain cake formula was used:

Hydrogenated fat	56	gm.
Egg	48	gm.
Sugar	150	gm.
Salt	3	gm.
Water	102	gm.

Vanilla	5 ml.
Dried whole milk	20 gm.
Tartrate baking powder	4.5 gm.
Cake flour	142 gm.

For the glass pan and for the larger steel pan this formula was doubled.

Combining the Ingredients: All ingredients except the eggs were incubated at 25°C.(77°F.). The eggs were stored at refrigerator temperature and warmed to mixing temperature just before they were used. Mixing operations were carried out at 25°C.(77°F.).

The batter was mixed with an electric mixer having ten speeds.[1] The fat, sugar, salt, egg, and 12 ml. of the water (24 ml. when the formula was doubled for the larger pans) were creamed at Speed 6 for six minutes. One-half of the sifted dry ingredients (flour, dried milk, and baking powder) and one-half the liquid were combined with the creamed mixture, with the mixer running at Speed 1 for 25 seconds. The second portions of both the dry ingredients and the liquid were added and these were incorporated at Speed 1 for 25 seconds. The sides of the mixing bowl were scraped and mixing continued at Speed 1 for an additional 25 seconds, making a total of 75 seconds' mixing time after the first portions of liquid and of dry ingredients were added to the creamed mixture.

Immediately after mixing, the batter was weighed into the pan being tested. For the smaller pans with a capacity of 680 ml., 250 gm. of batter were used, while for the larger pans 625 gm. were used.

Baking: Cakes were baked individually as soon as the batter was weighed into the pan. Care was taken to place the pan of batter in the center of the oven. Baking was done in an electric oven having a control with three levels of heat intensity and equipped with an interior light and a glass window. The cakes were baked at 185°C.(365°F.). After an initial adjustment period which lasted from one to four minutes after the oven door was closed on the pan of cake batter, the temperature of the oven returned to 185°C.(365°F.). During the baking period the oven temperature seldom varied as much 1°C.

Measuring Heat Penetration: To measure the rise in internal temperature of the cake batter during the baking period, a calibrated, short-stemmed, mercury-glass thermometer with a short bulb was used. The thermometer was held in position in the batter by a small metal support which rested on either side of the pan. The thermometer was adjusted so that its bulb was centered in the batter. The initial temperature of the batter was recorded before placing the pan of batter in the oven, and temperature readings were taken through the glass window at one-minute intervals during the baking period. Obviously, timing the baking period was not feasible as a means of having all cakes baked to a comparable degree of doneness, because of differences inherent in the pans. Instead, as a criterion of doneness, the cakes were baked to a maximum internal temperature and for four minutes longer.

At the end of the baking period the cakes were removed from the oven and allowed to cool in the pans to 60°C.(140°F.). They were then

[1] Kitchen Aid, Model K4-B.

removed from the pans and placed on a rack to continue cooling to room temperature. Each cake was then wrapped in waxed paper, and stored in a metal box with a tight-fitting cover.

Measuring Volumes of Finished Cakes: Volumes of the baked cakes were taken by seed displacement on the day following baking. Dusting the surface of each cake lightly with cornstarch and removing the excess before measuring the volume helped to keep the flaxseeds from sticking to the surface of the cake.

Scoring the Cakes: Approximately 24 hours after they were baked the cakes were rated subjectively for appearance and for eating qualities. The method of rank scoring was used, the judges listing the cakes in descending order for each characteristic listed on the score card.

The uncut cakes were first rated for two external qualities—shape of top and color of crust. Cut slices of the cakes were then ranked for eating qualities. The latter included texture, lightness, moistness, tenderness, and over-all desirability of the cake crumb.

Statistical Treatment:[2] Data for both the objective measurements and the subjective scores were analyzed statistically, with the exception of the scores for the cakes in Series II, where the results were so clear-cut that statistical treatment was unnecessary.

RESULTS AND DISCUSSION

Heat Penetration: Series I. As the heat penetration curves (Fig. 1) and the figures for baking times (Table 2) show, the eight small metal baking pans in Series I fall roughly into one of two groups—those that were fast, and those that were slow, to bake. The faster-baking pans included those made from steel, japanned iron, anodized aluminum, and iron; while the bright aluminum, the tinned iron, the copper, and the stainless steel were relatively slow-baking pans. Statistical treatment of the data showed that difference in baking times between the two groups was significant. The minimum significant difference between any two totals at the five per cent level is included (Table 2).

Comparisons within the group of faster-baking pans show that cakes in the steel, the japanned iron, and the anodized aluminum pans baked significantly faster than did those in the iron pan. Cakes in the steel pan did not bake significantly faster than did those in the japanned iron pan; nor did those in the japanned iron pan bake significantly faster than those in the anodized aluminum pan. In fact, the difference between the steel pan, which was the fastest-baking pan, and the anodized aluminum pan, which rated third, barely exceeded the minimum significant difference at the five per cent level.

In the slow-baking group, both the bright aluminum and the tinned iron pans baked significantly faster than did either the stainless steel or the copper pan. Baking time for the bright aluminum pan did not differ significantly from that of the tinned iron pan, nor did that for the stainless steel differ significantly from that of the copper pan.

[2] Statistical work was done under the direction of Dr. Jerome C. R. Li, Department of Mathematics, Oregon State College, Corvallis, Oregon.

In Series II baking time for the glass pan proved to be significantly longer than that for a pan of the same dimensions made from steel ($F = 63.16$ with one and seven degrees of freedom). (See Table 3.) The smaller pan made from the same material was one of the three fastest-baking metal pans in Series I.

Littleton and Phillips (1932), using two glass baking utensils, one untreated, highly emissive, and transmitting approximately 95 per cent of the radiant heat striking it, the other silvered and reflecting 98 per cent of the radiant heat striking it, reported that approximately two-thirds of the heating accomplished in the electric oven they used was due to radiation, while the remainder was due to convection. Transfer of heat throughout the oven by conduction is negligible because of the low thermal

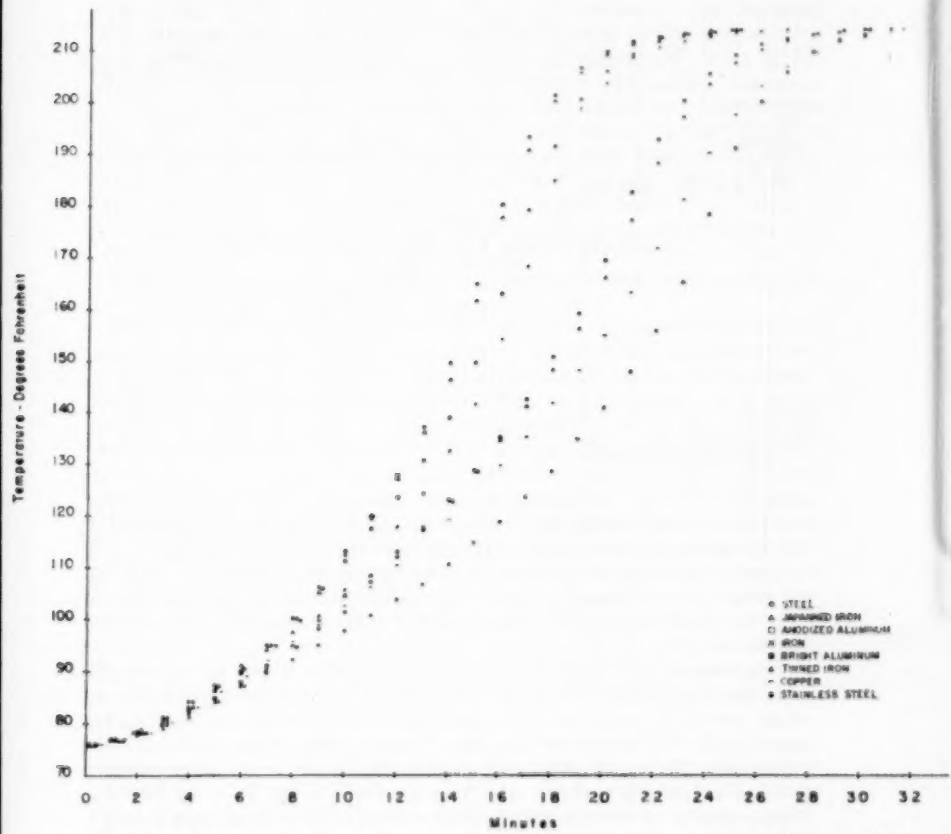

FIG. 1. Heat penetration in cakes made with fat baked in eight metal pans (based on eight replications).

TABLE 2

Observations on Cakes Made With Fat, Baked in Pans Made From Different Metals [1]

Pan	Baking time			Time to cool to 60°C. (140°F.)		Weight loss		Volume	
	Total	Av.	Rank	Total	Rank	Total	Rank	Av.	Rank
	min.	*min.*		*min.*		*gm.*		*ml.*	
Steel	215	26.8	1	223	3	123.9	3	1,515.6	3
Japanned iron	217	27.1	2	213	2	124.2	2	1,520.8	1
Anodized aluminum	221	27.6	3	206	1	121.4	5	1,517.5	2
Sheet iron	228	28.5	4	234	4	125.5	1	1,506.7	4
Bright aluminum	252	31.5	5	237	5	113.8	8	1,481.7	7
Tinned iron	256	32.0	6	238	6	118.5	6	1,494.6	5
Stainless steel	272	34.0	7	292	8	122.4	4	1,478.8	8
Copper	273	34.1	8	241	7	117.0	7	1,483.8	6
Minimum significant difference at five per cent level	5.5			9		4.3		7.9	

[1] Based on eight replications.

TABLE 3

Observations on Cakes Made With Fat, Baked in Glass and in Steel Pans [1]

Pan	Baking time		Time to cool to 60°C. (140°F.)	Weight loss	Volume
	Total	Av.	Total	Total	Av.
	min.	*min.*	*min.*	*gm.*	*ml.*
Glass	304	38	423	381.9	1,569
Steel	280	35	291	435.1	1,572

[1] Based on eight replications.

conductivity of air. Of course, heat brought to a utensil in the oven by convection currents must pass through the walls of the pan by conduction. But the thermal conductivity of the material from which a baking utensil is made is of minor importance in determining its heating efficiency in the oven, not only because of the high proportion of radiant heat in an oven but because of other factors which influence transfer of heat by conduction.

The transfer by conduction of heat brought to a pan by convection currents depends not only upon the conductivity of the material from which the pan is made but also upon the extent to which the heat makes contact with the surface of the pan, Austin (1941). The main interference to the passage of heat from the circulating air in the oven through the pan and to the contents of a baking utensil is the more or less stationary film of air on the outside of the utensil. Heat must pass through such films by conduction, a slow process; hence, these films offer so much

resistance to passage of heat they nullify any effects the high thermal conductivity of the material might have. This is especially true where metals are relatively thin, as they are in baking utensils. For the same reason, differences in conductivity, owing to small differences that exist in thickness of baking utensils, are insignificant compared with the effect of films upon heat transfer by conduction.

Because of the interference of films to the flow of heat through materials of potentially high thermal conductivity, and because of the relatively high proportion of radiant heat available in an oven, the emissivity of a baking utensil is the important factor in determining its efficiency in utilizing oven heat.

Materials with rough or dull surfaces absorb and transmit radiant heat, while those with bright surfaces, being good reflectors, are poor transmitters of radiant heat, Austin (1941). Metals, particularly those with a high polish, do not utilize radiant heat efficiently. This accounts for the longer baking time for the stainless steel and for the copper pans used in this study, both of which had a very high polish, and for the bright aluminum and the tinned iron pans, both with a high polish.

In contrast to metals, non-metals, as a rule, absorb and emit radiant heat more readily, so that oxide either on the surface or throughout a metal increases its emissivity. The surface of the sheet iron pan, an appreciably faster-baking pan than the tinned iron one, was covered with a dark, uneven coat of iron oxide and had a rough, almost pitted surface. The sheet iron pan was three times thicker than the tinned iron pan, yet cakes baked in the sheet iron pan in three and one-half minutes less, on the average, than in the tinned iron pan, showing the relative unimportance of thickness of the utensil upon its baking efficiency.

The anodized and bright aluminum pans offer an interesting contrast. The former is made from an aluminum alloy which has been electrolytically oxidized, a process which gives a hard, dull surface to the metal. Cakes in the anodized aluminum pan baked, on the average, in approximately four minutes' less time than did those baked in the bright aluminum pan, a reduction in baking time of 12.3 per cent. Comparing the japanned iron pan with the tinned iron of practically the same thickness (0.24 mm. versus 0.25 mm.), the japanned iron pan proved to be appreciably more efficient. In this case the dark lacquer on the surface of the iron increased the emissivity of the metal, so that cakes baked on the average in five minutes' less time in the japanned iron pan than in the tinned iron one, a reduction in baking time of 15.6 per cent. The steel pan, which was coated with black iron oxide and had a rough surface, was highly emissive, although it did not bake significantly faster than the japanned iron pan, the surface of which, although shiny, was darker. In Series I the faster-baking pans thus proved to be those with a dark color and/or a dull finish, factors which increased their emissivity.

Baking time for cakes baked in the steel pan in Series II averaged three minutes less than that for cakes baked in glass, a reduction in baking time of eight per cent. Thus, the glass pan which is known to be highly emissive, proved less efficient than a pan of the same size and shape made from the dark, dull steel.

Weight Loss During Baking and Cooling to 60°C.(140°F.): It has been recommended that cakes made with fat be cooled in the pans to a temperature of 60°C.(140°F.), to give the cake crumb sufficient rigidity to withstand handling without damage to its structure. Cakes in this study required approximately as much time to cool to 60°C. as they did to bake (see Table 2). The one exception was the glass pan, with a cooling period appreciably longer than the baking period.

Cakes baked in the different pans showed differences in weight loss during baking and subsequent cooling to 60°C. Analysis of variance showed these differences were significant. In Series I cakes baked in the four faster-baking pans and in the stainless steel one lost significantly more weight during baking and cooling to 60°C. than did those baked in the remaining three slower-baking pans with the longer baking times.

Presumably, weight losses during baking would be influenced indirectly by the effect of the pan on length of the baking period, and perhaps by other baking effects inherent in the various pans. To separate the effects of length of baking period from other baking pan effects, the data on weight losses during baking were submitted to analysis of co-variance. When the effect of the baking time was eliminated, the effect of the pan on weight loss during baking was significant ($F = 12.99$ with seven and 48 degrees of freedom). While those pans which required longer baking times seemed to cause less weight loss ($r = -0.6070$), when the effect of the pan was removed the longer the cakes were baked the greater the weight loss ($r = 0.6163$).

In Series II the weight loss for cakes baked in the glass pan was significantly greater than that for cakes baked in the steel pan ($F = 71.91$ with one and seven degrees of freedom).

Volumes: In Series I the japanned iron, dull aluminum, steel, and iron pans yielded cakes with significantly larger volumes than did the tinned iron, bright aluminum, copper, or stainless steel pans (Table 2). Cakes baked in the japanned iron, anodized aluminum, or steel pans did not differ significantly in volume, but cakes baked in the iron pan were significantly smaller in volume than those baked in the three faster-baking pans. In the slow-baking group, cakes baked in the tinned iron pan were significantly larger than those baked in the bright aluminum, stainless steel, or copper pans. Volumes of cakes baked in the last three pans mentioned did not differ significantly. Thus, the more efficient metal pans produced cakes with appreciably larger volumes than did the less efficient pans.

In Series II differences in volumes between cakes baked in the glass and those baked in the steel pan were not significant ($F = 0.01$ with one and seven degrees of freedom).

Stone (1934) has reported that the volume of a plain cake increases with increasing oven temperature up to 185°C.(365°F.), while Cook (1938), using chocolate cake, reported an increase in volume with increasing baking temperature up to 225°C.(437°F.). With the chocolate cakes, at least, the increased baked volume was attributed in part to decreasing shrinkage during the last of the baking and subsequent cooling period as the oven temperature increased up to 195°C.(383°F.), at which temperature no

TABLE 4

Average Normal Scores Based on Subjective Ratings for Cakes Made with Fat, Baked in Pans Made From Different Metals[1]

Pan	Shape of top		Color of crust		Lightness		Grain		Texture		Moistness		Over-all quality of crumb	
	Av. score	Final rank	Av. score	Final rank	Av. score	Final rank	Av. score	Final rank	Av. score	Final rank	Av. score	Final rank	Av. score	Final rank
Steel	−0.708	7	−0.981	8	0.371	3	0.593	1	0.614	2	0.580	2	0.597	1
Japanned iron	−0.597	6	−0.905	7	0.441	2	0.465	2	0.615	1	0.616	1	0.531	2
Anodized aluminum	−0.159	5	−0.388	5	0.683	1	0.427	3	0.382	3	0.228	4	0.394	3
Sheet iron	−0.714	8	−0.520	6	0.138	4	0.323	4	0.169	4	0.273	3	0.358	4
Bright aluminum	0.060	4	0.817	1	−0.333	6	−0.256	5	−0.459	7	−0.510	8	−0.473	7
Tinned iron	0.478	3	0.677	2	−0.277	5	−0.524	8	−0.562	8	−0.456	7	−0.538	8
Stainless steel	0.776	2	0.626	4	−0.449	7	−0.507	6	−0.455	6	−0.430	6	−0.425	5
Copper	0.864	1	0.675	3	−0.574	8	−0.520	7	−0.304	5	−0.300	5	−0.443	6
Minimum significant difference at the five-per cent level	0.5019		0.3488		0.5462		0.3558		0.5775		0.5769		0.5660	

[1] Based on five replications.

shrinkage was observed. The data here reported show that at a constant oven temperature of 185°C.(365°F.) the efficiency of the baking utensil can influence the volume of the baked cake.

To see if the differences in volumes of the cakes baked in the different pans could be related to differences in baking times or to other baking effects inherent in the different pans, data for the pans in Series I were submitted to analysis of co-variance. When the effect of the baking time was eliminated, the pan itself still had a significant effect on the volume of the cake ($F = 5.74$ with seven and 48 degrees of freedom). The pan which required the longer baking time gave a smaller volume ($r = -0.9479$). On the other hand, when the effect of the pan was eliminated there was no correlation between baking time and cake volume ($r = 0.0797$). Data here presented indicate that with an increase in efficiency of the baking utensil there is a favorable effect on cake volume similar to that obtained by elevating the baking temperature, but that this is a pan effect apart from any indirect effect of the pan upon length of baking time.

Subjective Scores: Three judges rated each of five replications from each cake pan for the subjective scores. On the day following baking each judge was asked to rank the eight uncut cakes for (1) shape of top and (2) color of crust. Cut slices from each of the cakes were then ranked for eating qualities. The latter included lightness, grain, texture, moistness, and over-all desirability of the cake crumb. For analysis of variance, ranks for the cakes in Series I were transformed into normal scores, using the table of Fisher and Yates (1938). Statistical treatment of the data for the subjective scores of these cakes is summarized (Table 4).

Number of times each of the two cakes in Series II was ranked best for each item on the score card is summarized (Table 5). As each of the three judges ranked five sets of cakes, the maximum number of top ratings

TABLE 5

Number of Times Cakes Were Preferred by Judges[1]

Pan	Shape of top	Color of crust	Lightness	Grain	Texture	Over-all rank
Glass	13	1	4	5	5	5
Steel	2	13	9	9	9	10

[1] Based on five replications for each of three judges.

possible for each cake was fifteen. In two instances a judge was unable to detect any difference in lightness between the two cakes and in one instance a judge was unable to tell a difference in color of the crust, grain, and texture of the two cakes.

1. Appearance of Cakes

(a) *Shape of Top:* Cakes with flat or slightly rounded tops are considered more desirable than those with excessively rounded or peaked tops. In Series I of this study the shapes of the tops of cakes baked in the copper, stainless steel, tinned iron, and bright aluminum pans were rated higher than those baked in the anodized aluminum, japanned iron, steel, or iron pans. Cakes in the latter group tended to be excessively humped or peaked. Thus, the faster-baking pans produced an effect on

the shape of the top of the cake comparable to that of elevating the baking temperature. In the slow-baking group, cakes baked in the copper, stainless steel, and tin pans were not rated significantly different in regard to shape of the top. Scores for cakes baked in the copper and in the stainless steel pans were significantly higher than were those for cakes baked in the bright aluminum pan, while scores for those baked in the tinned iron pan were not. In the group of four faster-baking pans scores for shape of top of the cakes baked in the anodized aluminum pan were significantly higher than were those for cakes baked in either the steel or the iron pan. Scores for cakes baked in the steel, japanned iron, and iron pans did not differ significantly. Bright aluminum in the group of slower-baking pans did not produce cakes that rated significantly better as to shape of top than did the faster-baking anodized aluminum pan.

In Series II the shapes of the tops of cakes baked in the glass pan were rated higher than were those of cakes baked in the steel pan.

(b) *Color of Crust:* Other things being equal, cakes with a uniform golden-brown crust are preferred. In this study the slower-baking pans in Series I produced cakes with significantly higher scores in this respect than did the four faster-baking pans. Cakes baked in the latter were either excessively or unevenly browned, with a tendency for sides of the cakes to be much browner than tops. Scores for color of the crust of cakes from the bright aluminum, tin, copper, and stainless steel pans did not differ significantly. In the faster-baking group scores for color of crust of cakes from the anodized aluminum and the iron pans were not significantly different, nor were those from the japanned iron and the steel pans. However, cakes baked in either the dull aluminum or the iron pan scored significantly higher as to color of crust than did those baked in the japanned iron or in the steel pan.

In Series II the color of the crust of cakes baked in the steel pan was rated higher than was that of cakes baked in the glass pan. Crusts of cakes baked in the latter were considerably thicker, especially at the corners, and appeared almost scorched along the top edges.

In Series I, then, those cakes baked in the faster-baking metal pans had larger volumes, but with one exception were inferior in external appearance to cakes baked in the slower-baking pans. When cakes baked in a glass pan were compared with those baked in one of the faster-baking metal pans, results were not consistent with those of Series I. While the steel pan had a shorter baking time and produced cakes that rated lower for shape of top, color of crust was rated higher than for those cakes baked in the glass pan. There was no difference in the volume of the cakes from the two pans.

2. Crumb Quality

(a) *Lightness:* In Series I three of the four faster-baking pans (anodized aluminum, japanned iron, and steel) produced cakes rated significantly lighter than cakes from the four slower-baking pans. It should be recalled that cakes from these same three metal pans had significantly larger volumes than cakes from any other pans in the series. Cakes from the iron pan were not rated significantly lighter than those from the tin

and from the bright aluminum pans, but were rated significantly lighter than those from the stainless steel and the copper pans. Differences in lightness of cakes from pans within the slower-baking group were not significant.

In Series II cakes baked in the steel pan were rated lighter than those baked in the glass pan.

(b) *Grain:* In Series I cakes baked in the faster-baking pans rated significantly higher as to fineness of cells and thinness of cell walls than did those baked in the slower-baking pans. Differences in scores among pans within each of the two groups were not significant.

In Series II cakes baked in the steel pan rated higher with respect to grain than did those baked in glass.

(c) *Texture:* When cakes baked in the different pans in Series I were compared for texture, those from the japanned iron, steel, and anodized aluminum pans were rated superior to those baked in any one of the four slower-baking pans. Cakes from the sheet iron pan were rated significantly superior in texture to those baked in the stainless steel, bright aluminum, and tinned iron pans, but not superior to those baked in the copper pan. Differences in texture among cakes within the two groups were not significant.

In Series II cakes baked in the steel pan rated better in texture than did those baked in glass.

(d) *Moistness:* In Series I cakes baked in the japanned iron and in the steel pans rated significantly higher in moistness than did cakes baked in any one of the four slower-baking pans. Cakes baked in the iron and in the anodized aluminum pans rated significantly higher in moistness than those baked in the stainless steel, the tin, or the bright aluminum pans, but not significantly higher than those baked in the copper pan. Differences in ratings for moistness of cakes within groups were not significant.

In Series II cakes baked in the steel pans were rated higher than those baked in the glass pan, in respect to moistness.

(e) *Over-all Rank:* In over-all desirability of crumb, cakes from the four faster-baking pans in Series I were rated significantly more desirable than those from the slower-baking pans. Differences in the scores for over-all desirability within groups were not significant.

In Series II the crumb of cakes baked in the steel pan was rated more desirable than that of cakes baked in glass.

Thus, the more efficient baking pans yielded cakes with crumb quality superior to that of cakes baked in the slower-baking pans, an effect similar to that obtained by elevating the baking temperature.

SUMMARY

Effects of using baking pans made from steel, japanned iron, anodized aluminum, sheet iron, bright aluminum, tinned iron, stainless steel, copper, and glass on heat penetration during baking and on quality of cakes made with fat have been studied.

The steel, japanned iron, anodized aluminum, and sheet iron were significantly faster-baking pans than were the bright aluminum, tinned iron,

stainless steel, or copper ones. Thus, the faster-baking metal pans were those which were dark or dull or both. The glass baking pan required a significantly longer baking period than did a steel pan of the same size and shape.

The four more efficient metal pans produced cakes with significantly greater volumes than did the four less efficient ones. Cakes baked in the steel pan did not differ significantly in volume from those baked in an oven-glass pan of the same size and shape.

A panel of judges rated the uncut cakes for appearance, and the crumb for eating quality. Shapes of the tops of cakes baked in three of the four less efficient metal pans were rated significantly higher than those of the four faster-baking metal pans. Shapes of tops of cakes baked in the glass pan were rated above those baked in the steel pan.

For degree and evenness of browning cakes baked in the bright aluminum, tinned iron, copper, and stainless steel pans were rated superior to those from the four faster-baking metal pans. The judges rated the brownness of cakes baked in the steel pan superior to that of cakes baked in glass.

For over-all crumb quality cakes baked in the faster-baking metal pans were rated significantly higher than those baked in the four slower-baking metal pans. The quality of the crumb of cakes baked in the steel pan was rated higher than that of cakes baked in glass.

Thus, the more efficient metal pans produced cakes with larger volumes and better crumb quality than did the less efficient pans, but at some sacrifice in the appearance of the cakes. The steel pan, which was more efficient than a similar pan made from glass, yielded cakes rated superior for crumb quality and for brownness of crust, but inferior in shape of top. There was no significant difference in the volumes of cakes baked in the glass and in the steel pans.

ACKNOWLEDGMENT

The author is indebted to Andrea Overman of the Department of Home Economics, Oregon Agricultural Experiment Station, for the use of laboratory facilities, as well as for helpful suggestions in carrying out this investigation. The writer also wishes to express appreciation to the following judges for their cooperation: Alta Garrison, Agnes Kolshorn, and Andrea Overman.

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COMPARISON OF THE QUALITY OF FRESHLY BAKED CAKES, THAWED FROZEN BAKED CAKES, AND CAKES PREPARED FROM BATTERS WHICH HAD BEEN FROZEN

ROYENE F. OWEN AND FRANCES O. VAN DUYNE
Department of Home Economics, University of Illinois, Urbana, Illinois

[Received for publication, December 27, 1949]

The increased production and use of home freezing units have aroused interest in the freezing of baked products, batters, and doughs. Because of the convenience, many homemakers are freezing and storing these foods for short periods. This investigation was undertaken to study the effect of freezing on the quality of plain shortened cakes, and to determine whether cakes baked before freezing differed from those prepared from batter which had been frozen. Freshly baked cakes, cakes baked from frozen or thawed frozen batter, and reheated frozen cakes were compared on the bases of volume, compressibility, and palatability. These comparisons were made after one, four, eight, 12, and 16 weeks of freezer storage. Tests were made two and 48 hours after baking or reheating. The cakes were tested after 48 hours, to learn whether those which had been frozen, or prepared from batters which had been frozen, retained their freshness as well as standard products after 48 hours.

REVIEW OF LITERATURE

Numerous recommendations have been made concerning the freezing of cake batters and baked cakes, but there are few reports of controlled experiments. Graul and Lowe (1947) prepared shortened cakes and batters, using either a hydrogenated lard or butter, and compared the quality of the thawed cakes and of cakes baked from the batters after storage at −18 and −23°C.(0 and −10°F.) for two, four, six, and eight months. After two months noticeable flavor, aroma, and color changes were observed in cakes or cake batters held at −18°C.(0°F.), while products stored at −23°C.(−10°F.) did not differ significantly from fresh cakes. Volume decreased in all frozen products, the mean volume of cakes stored at −18°C.(0°F.) being smaller than that of cakes held at the lower temperature. Frozen baked cakes had better volume and were considered more palatable than cakes baked from frozen batters. These workers found that when synthetic vanilla was added to the mix, an unpalatable flavor developed at both storage temperatures. This flavor was not present when no vanilla was used.

Meyer, Buckley, and Moore (1949) compared the volume and palatability of sponge and butter cakes frozen after baking and prepared from frozen batter and thawed frozen batter. Butter cakes baked before freezing maintained good quality throughout the nine-month test period, while the ones baked from thawed batter were equivalent to fresh in all respects during six months of freezer storage, after which a slight off-flavor was noted. Those prepared from frozen batter at intervals during seven months of freezer storage were considered to be of good quality,

although they tended to be more compact, slightly smaller in volume, and humped rather than rounded in shape. Attempts to identify the off-flavor which developed in the frozen batters after six months were not conclusive.

In both investigations, good quality was retained by frozen baked cakes. However, the quality of products baked from batters which had been frozen varied. Fenton and Darfler (1946) have recommended freezing either after baking or as batter, choice being determined by convenience and preference.

No reports concerning the keeping quality of cakes which have been frozen or prepared from frozen batters were found in the literature, but Fenton and Darfler (1946) state that frozen baked cakes seem to dry out more quickly after thawing than do freshly prepared and baked cakes.

EXPERIMENTAL PROCEDURE

Preparation of Cakes: Plain shortened cakes were prepared by a quick-mix recipe and method described by Redfield (1947), with two modifications. After converting the amounts of ingredients from standard measures to gm., the amounts were increased 50 per cent by weight, in order to have enough batter for two cakes from each mixing. The following ingredients were used: 337.5 gm. cake flour, 13 gm. sodium-aluminum-sulfate baking powder,[1] six gm. salt, 450 gm. sugar, 150 gm. hydrogenated fat,[2] 150 gm. mixed whole eggs, 366 gm. whole milk, and five ml. vanilla extract.

The dry ingredients were sifted together four times and placed in the large bowl of an electric mixer having 10 speeds.[3] The eggs, milk, and vanilla were combined. The fat and one cup of the liquid were then added to the dry ingredients, and mixed at Speed Three (medium speed) for two minutes. After scraping the bowl and beaters, the remaining liquid was added and mixed for two minutes at the same speed.

The batter was transferred to quart freezer containers with slip-on lids or to aluminum baking pans, eight by eight by two inches. Approximately 800 gm. of batter were weighed into each freezer container, and 600 ± 2 gm. of batter into each pan. All batters, whether fresh, frozen, or frozen and thawed, were baked 45 minutes at 191°C.(375°F.).

Batters for the cakes in Series I were mixed on nine days between February 2 and March 1. Each mixing provided batter for two of the following variations: freezing in a quart container (a), freezing in a pan (b), or immediate baking (c). In three successive mixings, the batter from the first was used for variations (a) and (b); from the second, for (b) and (c); and from the third, for (a) and (c). This distribution of pairs was continuous throughout the series. On each day that cakes and batters were prepared, one baked cake was tested while the other baked cakes were frozen.

Batters for the cakes in Series II were mixed on days when products from Series I were removed from the freezer for testing: 600 ± 2 gm. of

[1] Calumet.
[2] Crisco.
[3] Sunbeam Mixmaster.

batter were baked to give a standard cake and 600 ± 2 gm. transferred to a pan for freezing. These batters were prepared between March 8 and April 26.

Freezing and Freezer Storage: The batters in quart containers or in pans wrapped and heat-sealed in cellophane were placed in a freezer immediately after mixing and packaging. The baked cakes were cooled at room temperature for 90 minutes (10 minutes in pan, 20 minutes on rack, and 60 minutes while wrapped in wax paper). Then they were wrapped and heat-sealed in cellophane prior to freezing. The batters and cakes were frozen and stored in a chest-type home freezer [4] having a freezing compartment with a fan and two storage compartments. The temperature throughout the freezer varied between −21° and −15°C.(−5° and 5°F.). After approximately 24 hours, the frozen products were transferred to a storage compartment where they were held for one, four, eight, 12, and 16 weeks. After four or five intervals of freezer storage, four samples of each variation were removed.

Distribution and Treatment of Cake Batters and Cakes: In all, 112 cakes were tested. On each day that cakes and batters were prepared for freezing or removed from the freezer for testing, a standard cake was also prepared and judged. There were 41 of these standard cakes. The remaining 71 samples of batters and cakes were handled as follows:

Frozen batters for 20 cakes which had been stored in quart containers were thawed in the cartons at room temperature to an internal temperature of 17°C.(63°F.). For baking, 600 ± 2 gm. of batter were transfered to a baking pan.

Batters for 35 cakes had been frozen in baking pans. Of these, 20 (prepared in Series I) were thawed at room temperature, without removing the cellophane, to an internal temperature of 17°C.(63°F.), and baked. The remaining cakes (prepared in Series II) were baked without thawing after periods of one, four, eight, and 12 weeks.

Sixteen frozen baked cakes were thawed in their cellophane wrappings by reheating for 30 minutes at 149°C.(300°F.). The internal temperature of each cake was measured when it was removed from the oven. These cakes were not tested after one week of freezer storage.

Quality Testing: Volume, compressibility, and palatability were measured two and 48 hours after baking or reheating. Cakes prepared from fresh, frozen, or frozen and thawed batters were cooled 30 minutes (10 minutes in pan and 20 minutes on rack) and then wrapped in wax paper for approximately 90 minutes. Reheated frozen cakes were cooled in the cellophane wrapping for approximately two hours before testing.

Half of each cake was tested two hours after baking or reheating. The other half was wrapped in two thicknesses of wax paper and held in a bread box for testing after 48 hours.

Volume: Index to volume measurements were made, using a polar planimeter according to the directions of Platt and Kratz (1933).

Compressibility: Compressibility was determined with the compressometer used by Powers and Simpson (1947). This instrument has been modified by attaching a small electric motor which raises at a uniform

[4] Esco.

rate the platform holding the cake. Compressibility readings were made on four slices from each half of cake, using slices alternate to those judged for palatability.

Palatability: Four judges rated the cakes for palatability, using the score sheet for plain cake described in the U. S. Department of Agriculture Farmers' Bulletin No. 1775 (1937). Since the cakes were to be judged after storage in a freezing unit, and after holding for two or 48 hours at room temperature, the score sheets were modified so that flavor, which originally received 30 points on the basis of 100, received 20 points, while freshness received 10. Ratings of one, two, three, four, and five (designating very poor, poor, fair, good, and very good, respectively) were indicated by the judges and these values weighted to give the final score. The judges also gave the cakes a general desirability score, using the same rating scale. Each judge received slices for tasting from the same position in each half of cake.

RESULTS AND DISCUSSION

The cake formula used in this study produced a light, tender, sweet cake. Because of the relatively large amount of sugar, the cakes were rated down by some judges because of the slightly "sugary" crust and sweet flavor. Weather conditions seemed to affect the external and internal characteristics of the cakes and probably caused some of the variations in volume and compressibility scores. The index to volume measurements of individual standard cakes two hours after baking ranged from 11.97 to 14.73 square inches, and the compressibility readings ranged from 50 to 81 gm. Because of these variations, mean values for volume, compressibility, and palatability of the frozen products were compared to those for standard cakes baked on the days cakes and batters were put into the freezer, and also on days these were tested after removal from the freezing unit (Table 1). Differences between mean scores for these tests on standard cakes were not statistically significant.[5]

During freezer storage there were no significant weight changes. Stored products showed a mean weight gain of 1.1 gm. No changes in color or appearance due to drying out were noted on the surface of the batter. Graul and Lowe (1947) report only small weight losses, one-half gm. or below, during the first four months of freezer storage. Surface changes which they describe occurred after six months.

The mean time required for thawing batters frozen in quart containers was 4.75 hours at room temperature. Batters frozen in baking pans required approximately 1.75 hours. These were thawed during the spring months when the temperature in the laboratory varied between 24 and 27°C.(75 and 81°F.). Frozen baked cakes reached an internal temperature of 50°C. ± 3°(122°F. ± 5°) after 30 minutes in an oven at 149°C. (300°F.). This procedure for thawing was adopted because it was consid-

[5] Standard deviation of the means $= S\bar{X} = \sqrt{\frac{\Sigma x^2}{n(n-1)}}$

in which x = deviation of each observation from mean,
n = number of observations.

Standard error of the difference between means $= \sqrt{(S\bar{X}_1)^2 + (S\bar{X}_2)^2}$

A difference is considered significant when it is equal to or greater than three times the standard error of the difference between means.

TABLE 1

Mean Values for Quality Factors of Cakes Tested Two Hours After Baking or Reheating

Treatment of frozen batters or cakes	Freezer storage	Cakes	Index to volume measurements			Compressibility			Palatability			General desirability		
			Standard[1]	Frozen	Standard[2]	Standard[1]	Frozen	Standard[2]	Standard[1]	Frozen	Standard[2]	Standard[1]	Frozen	Standard[2]
	wk.	*no.*	*sq. in.*	*sq. in.*	*sq. in.*	*gm.*	*gm.*	*gm.*						
Batters frozen in cartons, thawed before baking	1	4	14.18	13.72	13.85	64	65	64	93	93	92	4.5	4.6	4.6
	4	4	13.76	13.38	13.80	58	63	66	92	90	92	4.6	4.3	4.5
	8	4	13.76	13.36	14.10	58	72	64	92	92	94	4.6	4.3	4.7
	12	4	14.05	13.62	14.06	62	70	53	94	90	94	4.5	4.2	4.6
	16	4	14.14	13.93	14.15[5]	64	62	64[5]	93	88	89[5]	4.5	4.4[5]	4.3[5]
Means and standard deviations of the means	....	....	13.98 ±.083	13.60[3] ±.080	13.98 ±.101	62 ±1.3	66 ±1.6	62 ±1.6	93 ±.6	91 ±.6	93 ±.7	4.5 ±.04	4.4 ±.05	4.5 ±.05
Batters frozen in pans, thawed before baking	1	4	14.32	13.42	13.64	66	71	61	93	90	91	4.5	4.4	4.5
	4	4	13.89	13.49	13.74	57	60	68	92	90	92	4.5	4.3	4.5
	8	4	13.76	13.31	14.13	58	72	62	92	92	94	4.6	4.4	4.6
	12	4	14.23	13.08	14.02	65	74	60	94	88[6]	92	4.5	4.2[6]	4.4
	16	4	14.23	12.86	14.15[6]	65	74	64[6]	94	86[6]	89[6]	4.5	4.2[6]	4.3[6]
Means and standard deviations of the means	....	....	14.09 ±.07[7]	13.23[3,4] ±.100	13.92 ±.110	62 ±1.3	70[3] ±1.6	63 ±1.8	93 ±.7	89[3,4] ±.8	92 ±.6	4.5 ±.04	4.3[3] ±.04	4.5 ±.05
Batters frozen in pans, baked without preliminary thawing	1	4	14.06	13.41	13.73	62	67	72	93	92	93	4.4	4.5	4.5
	4	4	14.02	12.95	14.22	64	65	66	95	92	92	4.6	4.5	4.4
	8	4	13.78	12.91	13.69	69	70	62	93	88	92	4.3	4.3	4.6
	12	3	13.84	12.84	12.94	67	73	71	94	86	85	4.7	4.0	4.1
Means and standard deviations of the means	....	....	13.93 ±.100	13.04[3] ±.099	13.69 ±.223	66 ±1.7	68 ±1.1	67 ±1.9	94 ±.4	89[3] ±.8	91 ±1.1	4.5 ±.05	4.3 ±.07	4.4 ±.08
Baked, frozen, reheated cakes	4	4	14.18	13.48	13.53	64	64	70	93	88	92	4.5	4.2	4.5
	8	4	13.87	13.27	14.14	59	62	59	93	87	94	4.6	4.0	4.5
	12	4	13.76	13.26	14.16	58	64	56	92	86	94	4.6	4.1	4.6
	16	4	13.86	13.27	14.58[6]	60	66	62[6]	92	84	89[6]	4.6	4.1	4.2[6]
Means and standard deviations of the means	....	....	13.92 ±.085	13.32[3,4] ±.072	14.04 ±.126	61 ±1.3	64 ±1.3	61 ±2.1	92 ±.8	87[3,4] ±.8	93 ±.7	4.6 ±.04	4.1[3,4] ±.05	4.5 ±.07

[1] Standard cakes prepared on days cakes or batters were frozen. [2] Standard cakes prepared on days cakes or batters were tested. [3] Difference between mean values for frozen cakes and Standard 1 is significant. [4] Difference between mean values for frozen cakes and Standard 2 is significant. [5] Average for three cakes. [6] Average for two cakes.

ered practical and because heating to this temperature might have some beneficial effect on "freshness." Schoch and French (1947) present evidence that "refreshening" of staled bread and starch pastes occurs at 50°C.(122°F.). Reheating these cakes caused a mean weight loss of 5.5 gm., or approximately one per cent.

Quality of Cakes After Two Hours: Mean values for volume, compressibility, palatability, and general desirability are presented (Table 1). In general the mean volume, palatability, and general desirability scores were lower and the mean compressibility values higher, after freezing. However, differences were small, and significant only in cases indicated in the table.

Mean values for index to volume measurements of all frozen products were lower than corresponding figures for standard cakes. The mean volume of cakes prepared from batters frozen in cartons was greater than that of other frozen cakes, and that of cakes baked from frozen batter less. The difference between these mean volumes was significant, but other differences were not. In general, longer freezer storage caused decreases in volume. However, differences in mean volumes between various storage periods were not statistically significant.

Mean compressibility values were higher after freezing. With one exception, however, the differences were not significant. Products frozen in various ways did not differ significantly in compressibility. Usually compressibility was greater after longer freezer storage, but this trend was not consistent.

The mean palatability scores of cakes prepared from thawed batters frozen in cartons did not differ significantly from those of the standard products. Cakes prepared from batter frozen in the pans and thawed frozen baked cakes were significantly lower in palatability than standard cakes, with one exception. When the products from the freezer were compared, their mean palatability scores did not differ significantly. These cakes were rated good to high good, indicating that acceptable products were obtained by each freezing variation included. The mean general desirability scores support this observation. Decreases in mean palatability and general desirability scores with longer freezer storage were not significant during the 16 weeks of testing.

Statistical analysis of the mean values show that no method was superior in all respects. Except for volume, cakes prepared from batter frozen in cartons did not differ significantly from standard products. The slightly higher scores of these cakes indicate an advantage of this freezing technique. When batter was frozen in baking pans, mean scores were similar, whether the batter was baked while frozen or after thawing. Apparently, the batter thawed rapidly enough in the oven to allow baking to proceed normally. These results do not confirm those of Meyer, Buckley, and Moore (1949), who observed that cakes baked from frozen batters were not of as high quality as similar cakes prepared from thawed batters.

Reheated frozen baked cakes rated lower in palatability and general desirability than standard cakes and those prepared from batter which had been frozen. These results do not agree with those reported by Graul and Lowe (1947) and Meyer, Buckley, and Moore (1949), who found that the quality of frozen baked cakes after thawing was as high as or higher

than cakes prepared from batters stored in a freezing unit. In their investigations cakes were thawed at room temperature, while in the present study they were thawed in an oven. Comparison of the two methods of thawing would be necessary to determine whether thawing at room temperature is superior to reheating in an oven.

Quality After 48 Hours: The mean values for quality measures 48 hours after baking are presented (Table 2). These tests were included in an attempt to discover whether cakes which had been frozen or prepared from frozen batter staled more rapidly than freshly prepared and baked cakes. The difficulties of measuring staling are well known. Platt and Powers (1940) state that one of the best objective methods for following the staling of bread is by measuring the change in compressibility. Subjective ratings were also used to compare freshness of the cakes, and index to volume measurements were recorded.

Decreases in mean volume, palatability, and general desirability scores were similar for all cakes, as were also the increases in mean compressibility values. Thus, the quality ratings of the different products, although lower after 48 hours, followed the same general pattern that ratings did when the cakes were tested after two hours. The mean scores for freshness of standard and other cakes did not differ significantly, none of the cakes being considered particularly stale. However, the judges often indicated that the two-day-old cakes tasted dry. Platt (1930) found that bread from a richer formula staled at a slower rate than bread from a lean formula. Since the cakes prepared in this study were fairly rich, this may explain why the cakes were not rated stale even though the mean compressibility values were higher.

Analysis of Palatability Scores: A summary of the palatability scores is presented (Table 3). The lower mean palatability scores received by the frozen products were a result of slightly lower ratings for several qualities, rather than a sharp decrease in a single characteristic.

Surfaces of cakes prepared from frozen batters were rated lower because the "sugary" appearance of the crust was more pronounced. Parts of the crusts of the baked frozen cakes stuck to the cellophane when this was removed.

Differences between the mean volumes of the cakes measured objectively were not reflected in the subjective ratings. At no time were color changes noted, such as those reported by Graul and Lowe (1947) in cakes and batter prepared from hydrogenated lard and stored at −17°C.(0°F.).

No undesirable flavor changes occurred except in three cakes, which developed a flavor described by the judges as "fishy." These three cakes were prepared on the same day and frozen as batter, two in baking pans and one in a carton. They were tested after 12 and 16 weeks. Other products prepared the same day did not develop this objectionable flavor. However, the other batters were tested after one week, and the remainder were baked cakes. Since all ingredients except eggs and milk were purchased at the beginning of the study, it was felt that either the eggs or milk were of poor quality on this particular day, causing undesirable flavor changes in the batter after long storage. The scores of these cakes were not included in calculating the palatability means, following the rule for rejecting values found in Fales (1925).

TABLE 2

Mean Values for Quality Factors of Cakes Tested 48 Hours After Baking or Reheating

Treatment of frozen batters or cakes	Freezer storage	Cakes	Index to volume measurements			Compressibility			Palatability			General desirability		
			Standard[1]	Frozen	Standard[2]	Standard[1]	Frozen	Standard[2]	Standard[1]	Frozen	Standard[2]	Standard[1]	Frozen	Standard[2]
	wk.	*no.*	*sq. in.*	*sq. in.*	*sq. in.*	*gm.*	*gm.*	*gm.*						
Batters frozen in cartons, thawed before baking	1	4	12.72	12.34	12.50	82	85	83	86	86	86	4.0	4.0	4.0
	4	4	12.47	12.10	12.55	82	87	91	86	84	84	4.0	3.8	3.8
	8	4	12.47	12.30	12.95	82	92	87	86	86	86	4.0	3.9	3.8
	12	4	12.68	12.70	13.29	84	91	76	85	84	86	4.0	4.0	4.0
	16	4	12.73	13.10	12.82[5]	84	82	89[5]	85	82[5]	83[5]	4.0	3.7[5]	3.7[5]
Means and standard deviations of the means	....	....	12.61 ±.054	12.51 ±.117	12.82 ±.098	83 ±1.1	87 ±1.1	85 ±1.7	85 ±.3	85 ±.6	85 ±.4	4.0 ±.03	3.9 ±.05	3.9 ±.04
Batters frozen in pans, thawed before baking	1	4	12.76	12.30	12.45	84	85	81	86	84	84	4.0	3.8	4.0
	4	4	12.61	12.20	12.55	77	82	91	86	86	85	4.0	4.0	3.9
	8	4	12.47	12.31	12.84	82	85	86	86	86	88	4.0	3.9	4.0
	12	4	12.78	12.43	13.06	85	89	84	86	81[6]	85	4.0	3.8	4.0
	16	4	12.78	11.88	12.82[6]	85	84	89[6]	86	80[5]	83[5]	4.0	3.7	3.7
Means and standard deviations of the means	....	...	12.68 ±.048	12.22[3,4] ±.102	12.74 ±.082	82 ±1.1	85 ±1.2	86 ±1.6	86 ±.3	84 ±.7	85 ±.5	4.0 ±.03	3.8[3] ±.05	3.9 ±.13
Batters frozen in pans, baked without preliminary thawing	1	4	12.79	12.13	12.70	84	87	90	88	86	86	4.1	4.0	3.8
	4	4	12.84	11.98	13.14	86	90	84	86	84	87	3.8	3.8	4.0
	8	4	12.56	11.66	12.98	92	92	82	86	79	85	3.9	3.6	3.8
	12	3	12.83	11.57	11.68	89	91	95	85	74	77	3.9	3.4	3.4
Means and standard deviations of the means	....	....	12.75 ±.075	11.85[3,4] ±.113	12.69 ±.217	88 ±1.2	90 ±1.4	87 ±2.3	86 ±.6	81[3] ±1.5	84 ±1.4	3.9 ±.04	3.7 ±.08	3.8 ±.08
Baked, frozen, reheated cakes	4	4	12.72	12.28	12.60	82	88	92	86	83	84	4.0	3.8	3.8
	8	4	12.65	12.07	12.73	78	88	86	86	80	88	4.0	3.6	4.0
	12	4	12.47	12.32	13.27	82	94	79	86	76	85	4.0	3.4	4.0
	16	4	12.52	12.22	13.06[6]	82	85	86[6]	86	79	83[6]	4.0	3.4	3.8[6]
Means and standard deviations of the means	....	....	12.59 ±.068	12.24[3,4] ±.060	12.89 ±.103	81 ±1.5	89[3] ±1.4	85 ±2.3	86 ±.3	79[3,4] ±1.0	85 ±.8	4.0 ±.03	3.5[3,4] ±.06	3.9 ±.05

[1] Standard cakes prepared on days cakes were frozen. [2] Standard cakes prepared on days cakes were tested. [3] Difference between mean values for frozen cakes and Standard 1 is significant. [4] Difference between mean values for frozen cakes and Standard 2 is significant. [5] Average for three cakes. [6] Average for two cakes.

TABLE 3

Mean Palatability Ratings of Cakes

	Ratings	Shape	Surface	Volume	Texture	Grain	Color	Flavor	Freshness	Total score
	no.									
Possible score	----	10	10	10	20	10	10	20	10	100
Two hours after baking										
Standard cakes	147	9	9	9	18	8	10	20	10	93
Batters frozen in cartons, thawed before baking	78	9	8	9	17	9	10	19	10	91
Batters frozen in pans, thawed before baking	78	9	8	9	17	8	10	19	9	89
Batters frozen in pans, baked without preliminary thawing	57	8	8	9	17	9	10	19	10	90[1]
Baked, frozen, reheated cakes	60	9	7	8	17	8	10	18	9	86[1]
Forty-eight hours after baking										
Standard cakes	150	9	8	9	15	8	10	19	8	86
Batters frozen in cartons, thawed before baking	73	9	8	8	15	8	10	19	8	85
Batters frozen in pans, thawed before baking	68	8	8	8	15	8	10	18	8	83[1]
Batters frozen in pans, baked without preliminary thawing	55	8	8	8	14	8	10	17	7	80[1]
Baked, frozen, reheated cakes	55	9	8	8	14	8	10	17	7	81[1]

[1] Differences between these totals and those shown in Tables 1 and 2 are a result of rounding numbers in averaging.

The use of vanilla extract in cakes caused no undesirable changes, such as those described by Graul and Lowe (1947) when synthetic vanilla was added.

SUMMARY

Batters for plain shortened cakes were frozen in freezer containers and in baking pans; other portions of batter were baked and the cakes frozen. After freezer storage for one, four, eight, 12, and 16 weeks, volume and compressibility measurements and palatability ratings were made on cakes prepared from these batters and on reheated frozen cakes. Similar tests were made on freshly baked standard cakes. After 48 hours these tests were repeated on all products.

All cakes, when judged two hours after baking or reheating, rated from good to high good. Cakes baked from batter frozen and stored in cartons did not differ significantly from the standard products, except they were slightly smaller in volume. These cakes rated slightly higher than the other frozen products in palatability and general desirability and had a larger mean volume. However, in most cases the differences were not significant. Whether the batters frozen and stored in baking pans were thawed or not when the baking period started, similar mean values for volume and compressibility and mean scores for palatability and general desirability were obtained. Cakes baked before freezing and reheated to an internal temperature of 50°C. ± 3° (122°F. ± 5°) were scored lower on palatability and general desirability than were cakes baked from batter which had been frozen and stored.

Forty-eight hours after baking all cakes had decreased in volume, showed increases in mean compressibility readings, and were considered less palatable. However, cakes prepared from frozen batter and reheated frozen cakes apparently retained their "freshness" as well as did standard cakes.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Jean Chase Wolfe and Virginia Charles, who served with the authors as judges on the tasting panel throughout the study.

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